

**INVESTIGATIONS OF EVOLUTIONARY ARMS  
RACES AND HOST DIVERSITY IN AVIAN BROOD  
PARASITE SYSTEMS**

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## Abstract

Obligate brood parasites rely solely on other species, the hosts, to incubate their eggs and raise their offspring, which often reduces the host's reproductive output. This reproductive cost has led to the evolution of anti-parasite adaptations among hosts, which in turn, has led to better trickery by parasites, a process termed an evolutionary arms race. The objective of this thesis was to investigate host-parasite coevolutionary arms races to address questions of host-use diversity. Host diversity varies dramatically among brood-parasitic species, but reasons for variations in host-use among brood parasites are not well understood. In Chapter 2, I address questions on host diversity specifically, whereas I address questions about coevolutionary interaction between hosts and parasites in Chapters 3, 4 and 5 using two host-parasite systems, one in New Zealand and one in North America.

Chapter 2 investigates if host diversity is constrained by aggressive nest defence behaviour. I compared the nest defence behaviour of the exclusive host of the shining cuckoo *Chrysococcyx lucidus lucidus* on the main islands of New Zealand, the grey warbler *Gerygone igata*, to two other potentially suitable hosts that are not currently parasitised, the fantail *Rhipidura fuliginosa* and the silvereye *Zosterops lateralis*. The results suggest that grey warblers are as aggressive as fantails and silvereyes towards shining cuckoos at the nest and thus, host specialisation in shining cuckoos in New Zealand, at least, does not appear to be the result of nest-defence constraints imposed by potential but unused host species.

Chapter 3 investigates if red-winged blackbirds *Agelaius phoeniceus*, a species that typically accepts the eggs of parasites, recognises, as indicated by changes in incubation behaviour, when they have been parasitised by brown-headed cowbirds

*Molothrus ater*. Recognition without rejection suggests that rejection may be context-dependent but the results suggest that red-winged blackbirds do not recognise when their nests have been parasitised by brown-headed cowbirds, at least at the egg stage. This study was the first to investigate if hosts that almost invariably accept the eggs of parasites recognise when they have been parasitised.

Chapter 4 investigated the possibility of coevolutionary arms races occurring through olfactory channels in contrast to earlier work that focussed only on visual and auditory cues. Recent research has revealed that olfactory abilities in birds are more common than previously thought. Uropygial gland secretions are posited to be a key source of avian body odour and its composition has been found to vary among species and individuals as well as between the sexes. I compared gas-chromatography (GC-FID) traces of shining cuckoo preen wax to the GC-FID traces of the grey warbler, the only host of the shining cuckoo in mainland New Zealand, as well as the preen wax of seven other species for evidence of mimicry. Preliminary results suggest there is evidence for mimicry and the potential for odour-based nestling discrimination in grey warblers. Further tests recording the response of grey warblers to odour-manipulated nestlings are necessary.

Finally, in Chapter 5, I investigated the response of the song thrush *Turdus philomelos*, a species that rejects the eggs of the common cuckoo *Cuculus canorus* and conspecifics at intermediate and low frequencies, respectively, to nest-odour manipulations using the preen wax of conspecifics and heterospecifics. The results suggest song thrush do not use odour to assess the risk of parasitism at least as indicated in terms of changes in incubation behaviour. Investigations of the role of olfaction in avian brood parasite systems can provide a better understanding of brood-parasite coevolution. Only by considering all channels of communication can we be

sure to completely understand the coevolutionary dynamics between brood parasites and their hosts.

## **Chapter 1: General Introduction**

Being a specialist is a risky strategy (McKinney 1997). Specialisation can be a good strategy if the environment remains constant, but it can lead to extinction if conditions change (Poulin et al. 2006; Krüger et al. 2009). Host-specialisation among parasites seems especially risky because parasites rely on other species for their survival. Why some parasites specialise on a few species, whereas others parasitise many is not clear (Rothstein et al. 2002; Poulin et al. 2006). The coevolutionary interaction between obligate avian brood parasites and their host species provides an ideal system for studying the evolution of specialisation because host-use differs dramatically among different species of brood parasites (Rothstein 1990; Johnsgard 1997; Rothstein and Robinson 1998; Davies 2000).

Obligate brood parasitism is a rare reproductive strategy among birds and involves only about 1% of all c. 10,000 species (Davies 2000). Obligate brood parasites reproduce solely by laying their eggs in the nests of other species (i.e., hosts). The hosts then raise the parasitic young at the expense of their own reproductive success (Rothstein and Robinson 1998). The reproductive cost incurred by hosts when raising a brood parasite sets off a evolutionary arms race, where hosts evolve adaptations to mitigate the cost of parasitism which in turn select for improved adaptations in parasites to trick hosts into accepting their eggs and offspring. Theoretically, this reciprocal and antagonistic relationship can continue in perpetuity or until a stable evolutionary end point is reached (Janzen 1980; Futuyma 1998).

Despite being rare, obligate brood parasitism has evolved independently in birds at least seven times among five families: three times among the cuckoos (family Cuculidae), once in the cowbirds (family Icteridae), once in the finches (family Viduidae), once among the honeyguides (family Indicatoridae) and once in waterfowl (family Anatidae) (Payne 2005; Sorenson and Payne 2005). Obligate brood parasitism is known to be the reproductive strategy of 57 species of cuckoos (Cuculidae), five species of cowbirds (Icteridae), 17 species of honeyguides (Indicatoridae), 20 species of African finches (Viduidae), and one duck (Anatidae), the black-headed duck *Heteronetta atricapilla* (Davies 2000, 2011).

Host diversity among avian brood parasites varies from a few host species to more than 200 (Rothstein et al. 2002). For example, both the brown-headed cowbird *Molothrus ater* and shiny cowbird *M. bonariensis* parasitise over 200 species across their respective ranges, whereas the screaming cowbird *M. rufoaxillaris* uses only three host species (Friedmann and Kiff 1985; Lanyon 1992; Ortega 1998; Mahler et al. 2009). Likewise, host diversity varies extensively among the parasitic cuckoos. The common cuckoo *Cuculus canorus* is known to parasitise more than 100 host species whereas the long-tailed cuckoo *Urodynamis taitensis* is known to use only three (Davies 2000; Payne 2005). In honeyguides, host diversity varies from about 40 species in the greater honeyguide *Indicator indicator* to nine host species in the scaly-throated honeyguide *I. variegatus* (Short and Horne 2001). Among the African brood parasitic finches, the parasitic weaver *Anomalospiza imberbis* and pale-winged indigobird *Vidua wilsoni* each use five or six host species whereas most others use one or two (Johnsgard 1997). The eggs of the black-headed duck have been found in the nests of 14 species, although only three are known to have hatched ducklings (Weller 1968). However, even species with a large number of hosts typically use a

smaller number of preferred host species. For example, the common cuckoo parasitises only about 20 species regularly and at least 17 known host-specific *gentes* have evolved, each with highly specialised egg mimicry (Alvarez 1994; Moksnes and Røskoft 1995; Davies 2000; Edvardsen et al. 2001; Payne 2005; Antonov et al. 2007).

Differences in host diversity between different species of brood parasite may have evolved in response to variation in the ability of available host species to raise a parasite (Fraga 1998). Variability in the ability of sympatric species to act as hosts include differences in body size (Grim 2006), types of food provided to nestlings (Kleven et al. 1999), length of nestling period (Grim et al. 2003), nest accessibility, timing of breeding (De Mársico and Reboreda 2008a), abundance of host species (Langmore and Kilner 2007), breeding strategy (Astié and Reboreda 2009), and predation rate (Brooker and Brooker 1990; Avilés et al. 2006a; De Mársico and Reboreda 2008b). Furthermore, the ability of the parasite to compete with host young in host-tolerant parasites (Schuetz 2005a), and the cost of time and energy for evicting host eggs and nestlings in host-intolerant parasites (Anderson et al. 2009a; Grim et al. 2009a, b) may select for host preference and thus variation in host diversity.

Host diversity may be further constrained if suitable host species evolve well-developed adaptations that prevent successful parasitism and limit brood parasites to only those hosts with limited anti-parasite adaptations (Rothstein et al. 2002). Alternatively, host diversity may be maintained if parasites evolve counter-adaptations that trick hosts into raising parasites (Rothstein et al. 2002). For example, rejection of parasitic eggs is the best known defence used by hosts (Davies 2000). Visual cues are used by host species to detect parasitic eggs (Davies 2000; Avilés et al. 2010), with evidence that hosts can discriminate against parasitic eggs based on their shape (Underwood and Sealy 2006; Guigueno and Sealy 2009), size (Marchetti

2000), markings (Stoddard and Stevens 2010), and visible and ultra violet reflectance (Cherry et al. 2007a; Underwood and Sealy 2008; Avilés et al. 2010). In response, parasites may evolve egg mimicry to prevent hosts from rejecting their eggs (Brooke and Davies 1988; Moksnes and Røskft 1995; Honza et al. 2001) and female cuckoos may actually choose among host nests for best mimicry (Avilés et al. 2006b; Cherry et al. 2007b). Greater egg mimicry in the parasite should in turn select for improved discrimination of parasitic eggs in the host (Davies 2000). This reciprocal antagonistic interaction between parasites and their hosts has been termed an “evolutionary arms race” (Dawkins and Krebs 1979; Rothstein and Robinson 1998) and the outcome may determine the degree of host specialisation.

Recent research suggests coevolutionary arms races can continue beyond the egg stage because some host species discriminate against parasitic nestlings (Langmore et al. 2003; Schuetz 2005b; Soler 2009; Sato et al. 2010; Tokue and Ueda 2010; Delhey et al. 2011) and against parasitic fledglings (Rasmussen and Sealy 2006; De Mársico et al. 2012). For example, superb fairy-wrens *Malurus cyaneus* abandon nests containing a single Horsfield’s bronze-cuckoo *Chrysococcyx basalis* nestling, a brood parasite that specialises on fairy-wrens, or nests containing one of their own nestlings at a rate of 40% but deserted all nests containing a single shining bronze-cuckoo *C. lucidus plagosus* nestling, a rare fairy-wren parasite (Langmore et al. 2003). Large-billed gerygone *Gerygone magnirostris* have also been observed ejecting little-bronze cuckoo *C. minutillus* nestlings (Sato et al. 2010). Rejection by hosts has been suggested to select for the mimicry of nestling morphology and vocalisations in parasite species (McLean and Waas 1987; Schuetz 2005b; Anderson et al. 2009b; Soler 2009; Ranjard et al. 2010; Langmore et al. 2011). Host

discrimination, however, is not the only selective agent for counter-adaptations by parasites.

The division of host species among sympatric parasite species suggests competition among parasite species may also be a selective force for egg mimicry and crypsis (Brooker and Brooker 1990). Locally, most parasite species appear to use, almost exclusively, a host species or a group of host species that are used by no other parasite species, but some overlap does exist (Brooker and Booker 1989; Higuchi 1998; Ellison et al. 2006; Langmore and Kilner 2007). The partitioning of the “host” resources suggests that parasite species compete with each other for host nests (Brooker and Brooker 1990). Many parasite species remove an egg from the host nest immediately before laying their own egg (Davies 2000). It has been hypothesised that parasites may preferentially select a previously laid parasite egg or the most conspicuous egg to reduce the potential cost of competing with another parasite nestling (Brooker and Brooker 1990). Therefore, egg mimicry and crypsis may also be selected to lower the probability of rejection of parasitic eggs by competing parasites. However, Langmore and Kilner (2009) did not find evidence for this hypothesis because they recorded that the likelihood of removal by Horsfield’s bronze-cuckoo from splendid-fairy wren nests was approximately the same for non-mimetic eggs and mimetic eggs.

Reciprocal antagonistic interactions between parasites, their hosts, and their competitors can lead to the evolution of increasingly refined discriminatory abilities in hosts that can select for more precise host egg and nestling mimicry by parasites (Rothstein et al. 2002; Langmore et al. 2011). Genetic constraints, however, prevent an individual brood parasite from maintaining specific adaptations for all available host species and thus a species’ adaptations will be effective with fewer hosts over



time because egg and nestling appearances vary among host species (Rothstein et al. 2002). This results in parasites specialising on species with which they are most successful (Rothstein et al. 2002). Nevertheless, host diversity of some parasites has been maintained despite strong selection for specialisation through the formation of host races, such as in the gentes of the common cuckoo (Brooke and Davies 1988).

Some generalist brood parasite species are composed of individuals that specialise on one or a few host species. For example, common cuckoos are generalists at the species level (Davies 2000). However, genetic data and laying patterns reveal that individual female common cuckoos are specialists (Marchetti et al. 1998; Gibbs et al. 2000). These groups of females (i.e., gentes) share the same preference and well-developed egg mimicry for certain host species (Brooke and Davies 1988; Marchetti et al. 1998; Gibbs et al. 2000). Likewise, preference at the individual level has been found in screaming cowbirds that parasitise bay-winged cowbirds *Agelaioides badius*, their usual host species, and the chopi blackbird *Gnorimopsar chopi*, a new host to which some females have switched (Mahler et al. 2009). Brown-headed cowbirds have for a long time been thought to be host generalists (Rothstein and Robinson 1998) but genetic analyses have revealed that populations of this species may be composed of a combination of generalist and specialist individuals (Fleischer 1985; Alderson et al. 1999; Woolfenden et al. 2003; Strausberger and Ashley 2005; Ellison et al. 2006).

Reciprocal antagonistic interactions between parasite species and host species can lead to the local adaptation of parasite species for different host species (Thompson 2005; Ellison et al. 2006; Soler et al. 2009). The mechanisms leading to the reproductive isolation of host races are not well known (Brooke and Davies 1988) but genetic linkage of traits influencing host use and mate or habitat choice

(Hawthorne and Via 2001) and behavioural imprinting (Payne et al. 2000; Sorenson et al. 2003) have been suggested. This process suggests that the coevolutionary arms race leads from host races, to speciation, and finally to extinction (Davies 2000; Krüger et al. 2009). However, factors that influence the degree of association between host and mate choice, the speed of the coevolutionary arms race (Krüger et al. 2009), and the spread of adaptations within and among populations (Thompson 2005) can impede, restrict, or reverse the process of specialisation.

The strength of the association between mate and host choice can affect the rapidity of speciation and thus specialisation (Payne et al. 2000; Sorenson et al. 2003). For example, in the host-specific indigobirds, reproductive isolation is maintained through behavioural imprinting by both males and females on hosts (Sorenson et al. 2003). Imprinting by nestlings on the host nest in which they were raised determines female mate and host choice and male song in indigobirds. Females breed with males that sing the song of the host species that raised them (Sorenson et al. 2003). This mechanism maintains the reproductive isolation of host-specialist taxa and has facilitated rapid sympatric speciation when host switches have occurred (Payne et al. 2002; Sorenson et al. 2003). Alternatively, in common cuckoos, males mate with females irrespective of their host race (Marchetti et al. 1998), which apparently prevents the evolution of specialist species (Gibbs et al. 2000). Likewise, the polygamous breeding strategy of brown-headed cowbirds may prevent speciation among host-specific females (Woolfenden et al. 2002; but see Alderson et al. 1999).

Host-specific traits that are acquired after the parasite hatches and can be modified to improve reproductive success according to the host species are likely to impede the formation of genetically distinct lineages (Fanelli et al. 2005). The plasticity of host-specific adaptations has been demonstrated in Horsfield's bronze-

cuckoo (Langmore et al. 2008) and in the reed warbler *Acrocephalus scirpaceus* host race of the common cuckoo (Madden and Davies 2006), which modify their begging calls after hatching to maximise parental provisioning from different host species.

Relative host abundance may impede or facilitate the formation of host races (Soler et al. 2009). Equilibrium between being able to use the largest possible number of host species and being able to make the best use of each encountered host depends on the relative abundance of available host species (Soler et al. 2009). A specialist host strategy is selected when the benefits of specialising on an abundant host species outweigh the loss of efficiency when parasitising less abundant host species (Soler et al. 2009). For example, common cuckoos, on a local scale, have evolved host-specific egg mimicry for the host species with the highest population density and lowest population variability (Soler et al. 2009). Alternatively, heterogeneous host communities may select for the evolution of host-generalist strategies (Norton and Carpenter 1998).

The evolutionary trajectory of the interactions between parasite species and their host species may not necessarily be linear, as suggested above, but may instead be cyclic if parasite species switch between host species (Rothstein 2001). If the interaction between a parasite and a population of host species is linear, the long-term interaction between parasite species and a host species can have the following outcomes: (1) continued parasitism in the absence of the evolution of host defences because an equilibrium between the cost of rejection and the cost of acceptance has been achieved (Davies 2000; Rothstein 2001), or (2) the host species eventually evolves rejection behaviour that cannot be beat by the parasite (Honza et al. 2004; Lovász and Moskát 2004). Some parasite species, however, have been shown to switch or acquire new host species (Mermoz and Reboreda 1996; Cruz et al. 1998;

Rothstein 2001; Payne et al. 2002; Takasu et al. 2009) thus suggesting that some parasite-host systems follow a non-linear or cyclical evolutionary trajectory (Rothstein 2001).

Host switching is sustainable in the long-term only as long as host species lose their defences against parasitism when not parasitised and, that host species that are not parasitised lose their defences faster than parasitised host species acquire defences (Rothstein 2001). However, evidence for loss of defences by host species when not parasitised is equivocal and may depend on the cost of maintaining defences in the absence of parasitism (Cruz and Wiley 1989; Brooke et al. 1998; Rothstein 2001; Underwood et al. 2004; Hale and Briskie 2007). Therefore, the ability to switch host species is dependent on the availability of suitable host species that have not been parasitised or that have been parasitised but have subsequently lost their defences against parasitism (Cruz and Wiley 1989; Rothstein 2001) and the ability of a parasite species to efficiently parasitise new host species (Payne et al. 2002; Sorenson et al. 2003). Furthermore, host races may also form when parasites switch to new hosts and subsequently evolve adaptations to successfully parasitise them (Marchetti et al. 1998).

This thesis investigates interactions between brood parasites and their hosts with the goal of providing pieces of information that will improve our understanding of the evolutionary trajectory between brood parasites and their hosts. I took a two-pronged approach in this thesis. In Chapter 2 and Appendix 1, I specifically address questions about host specificity, using the shining cuckoo *Chrysococcyx lucidus* and the grey warbler *Gerygone igata* as a model parasite-host system. Then in Chapters 3, 4 and 5, I address questions about coevolutionary interactions between hosts and parasites, using two host-parasite systems, one in New Zealand and one in

North America, with the ultimate aim that one can better understand host diversity at a community level only by better understanding interactions within that community at the species level.

### ***Outline of thesis***

Chapter 2 investigates if host diversity is constrained by aggressive nest defence behaviour. The shining cuckoo is an extreme host specialist that parasitises the grey warbler exclusively on the main islands in New Zealand despite the presence of other species that are likely suitable as hosts. Host specialisation in the shining cuckoo is likely not the result of widespread egg rejection among available but unused hosts (Briskie 2003). I compared the nest defence behaviours of South Island fantails *Rhipidura fuliginosa fuliginosa* (hereafter fantails) and silvereyes *Zosterops lateralis*, two potentially suitable hosts that are not parasitised by shining cuckoos, to the nest defence behaviours of the grey warbler. I predicted that grey warblers should be less aggressive than fantails or silvereyes towards shining cuckoos if variations in the intensity of nest defence behaviours constrain host diversity. The results suggest that grey warblers are as aggressive as fantails and silvereyes towards shining cuckoos at the nest and thus do not support my prediction. Host specialisation among shining cuckoos in New Zealand, at least, does not appear to be the result of constraints imposed by aggressive behaviour by potential but unused hosts.

Chapter 3 investigates if hosts that almost invariably accept the eggs of parasites recognise when they have been parasitised. Changes in incubation behaviour in response to parasitism is a good indication that hosts recognise they have been

parasitised because it is correlated with rejection behaviour (Guigueno and Sealy 2012; Soler et al. 2012). However, this relationship is not perfect and some individuals apparently recognised the eggs of parasites because they changed their incubation behaviour in response to artificial parasitism but ultimately accepted the parasitic eggs (Antonov et al. 2007; Guigueno and Sealy 2012; Soler et al. 2012). To clarify the relationship between changes in incubation behaviour and recognition of parasitism, I compared the changes in incubation behaviour of red-winged blackbirds *Agelaius phoeniceus*, a species that almost invariably accepts the eggs of brown-headed cowbirds, to the changes in incubation behaviour of gray catbirds *Dumetella carolinensis*, a species that rejects the eggs of brown-headed cowbirds almost invariably, in response to the addition of an artificial cowbird egg to their clutch. I predicted that red-winged blackbirds, being accepters, would not change their behaviour in response to artificial parasitism, whereas, gray catbirds, being ejectors, would. The results suggest that red-winged blackbirds did not change their incubation behaviour and thus do not recognise when their nests have been parasitised by brown-headed cowbirds, at least at the egg stage.

As mentioned above, coevolution between hosts and parasites can lead to the evolution of increasingly specialised adaptations by parasites and increasingly better discriminatory abilities by hosts, however, genetic constraints may prevent an individual brood parasite from maintaining specific adaptations for all available host species and thus a species' adaptations will be effective with fewer hosts over time (Rothstein et al. 2002). An example of the result of the antagonistic coevolutionary interaction between brood parasites and their hosts is host egg mimicry by female common cuckoos which evolved in response to rejection of foreign eggs (Brooke and Davies 1988; Davies 2000; Avilés 2008; Cassey et al. 2008). In this thesis, mimicry is

used solely to describe similarities that have evolved as adaptations in response to discrimination by the host (sensu Grim 2005). Chapter 4 investigates how specialised a brood parasite can become by testing if there is evidence for host-odour mimicry in the shining cuckoo. Recent research has revealed the use of olfaction is widespread among birds and is used in a wide variety of behavioural contexts (Roper 1999; Hagelin and Jones 2007; Balthazart and Taziaux 2009). However, the use of olfaction by hosts to discriminate against parasite nestlings has not been investigated. Uropygial secretions (preen wax) are posited to be the key source of avian body odour. Determining if there is evidence for mimicry in preen wax composition is the first step in determining if grey warblers discriminate against nestlings with odours that are different from their own nestlings. In this chapter, I used gas chromatography flame ion detection (GC-FID) to compare the preen wax composition of the shining cuckoo to the preen wax composition of its host, the grey warbler, and to seven other sympatric species that may be suitable hosts but are not used by shining cuckoos. My objective was to assess similarity in GC-FID traces for evidence of mimicry of grey warbler odour by shining cuckoos. Visual assessment of the GC-FID traces suggest the preen wax composition of shining cuckoos and grey warblers are more similar to each other than to any of the other seven species I sampled. Mass spectra data are still required to properly align the gas chromatography data, which will be done before publication, but these results provide the first evidence for mimicry in an olfactory channel in any species of avian brood parasite.

Chapter 5 further explores the use of olfaction in host-parasite interactions. The body odour of a brood parasite left on the nest during a parasitism event may alert nest owners that their nest may be parasitised. Previous research has shown that blue tits *Cyanistes caeruleus* respond to the odour of predators at the nest (Amo et al.

2008) and dark-eyed juncos *Junco hyemalis* responded to the scent of conspecifics and heterospecifics at the nest (Whittaker et al. 2009) which suggests nest owners use changes in nest odour to adaptively change their behaviour. Song thrush *Turdus philomelos* reject the eggs of the common cuckoo and conspecifics at intermediate and low frequencies, respectively (Hale and Briskie 2007; Honza et al. 2007; Samas et al. 2011). I tested if song thrush use odour cues to assess the risk of parasitism. To do this, I tested if song thrush can detect, as determined by changes in their incubation behaviour, the preen wax of conspecifics and heterospecifics on their nest. I measured the incubation behaviour of song thrush on the day before, immediately after, and the day following the application of the preen wax of conspecifics and bellbirds *Anthornis melanura* to their nests. I used bellbird preen wax in trials in case song thrush cannot detect differences between their own odour and that of other song thrush. Bellbird composition differs remarkably from the preen wax of song thrush (see chapters 4 and 5). The results suggest that song thrush do not use odour to assess the risk of parasitism because they did not examine their nest contents more after nest odour manipulations and other measures of incubation behaviour remain unchanged in response to the addition of preen wax of either species.

Apart from the main chapters of my thesis, I also present 2 additional studies in the appendices that complement my general approach. In Appendix 1, I test the hypothesis that host diversity in the shining cuckoo is limited by the availability of suitable host species that do not possess effective anti-parasite defences (Fraga 1998; Rothstein et al. 2002). I do this by measuring the growth rate of two shining cuckoo nestlings fostered into fantail nests, a potentially suitable but non-used host, and comparing them to the growth rates of shining cuckoo nestlings raised by grey warblers. Shining cuckoos specialise on parasitising the nests of the grey warbler. No



reliable records exist for parasitism on any other species (Gill 1983). This chapter was relegated to the appendix because of the small sample sizes but it is included in the thesis because the data may be of interest to other researchers. The results suggest fantails are capable of raising a shining cuckoo nestling but they may desert them as they become older. Whether this indicates fantails may be unsuitable hosts requires further study.

In Appendix 2, I present the results of a study comparing the responses of red-winged blackbirds, in terms of changes in incubation behaviour, to real and artificial eggs. The difficulty of obtaining an amount of freshly laid parasitic eggs sufficient for scientific experiments has necessitated the use of artificial eggs by researchers. I compare the response of red-winged blackbirds to artificial and real cowbird eggs. The results suggest the response of red-winged blackbirds to artificial brown-headed cowbird eggs was different than the response to real brown-headed cowbird eggs, and thus the results from studies that only use artificial eggs should be viewed with caution unless the results can be confirmed with real eggs.

Finally, in Chapter 6, I provide a general discussion of my thesis. I start by reviewing the main findings and how these findings improve our understanding of variations in host diversity among avian brood parasites and our understanding of host-parasite evolutionary arms races. I finish by suggesting areas of future research that will extend the work I have done.

*Note on format of chapters:* The data chapters (i.e., chapters 2–5 and Appendix 1 and 2) of my thesis have been written up as independent manuscripts for publication and, as a result, some repetition was necessary. Each chapter is divided up into the following sections: ‘Abstract’, ‘Introduction’, ‘Methods’, ‘Results’ and ‘Discussion’

to correspond with the format of most scientific journals. Chapter 4 was written in collaboration with colleagues, however, I was the lead researcher and I will be the senior author on the resulting publication.

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## **Chapter 2: Does host nest defence behaviour constrain host diversity in avian brood parasites?**

### ***2.1 Abstract***

Host diversity varies dramatically among brood-parasitic species, but the reasons for the variations in host use among brood parasites are not well understood. As a first line of defence, some species defend their nests against adult brood parasites with aggressive nest defence behaviours such as striking and alarm calling. Other parasites, such as the yellow warbler *Setophaga petechia*, occasionally respond by sitting on the nest, which is believed to prevent the parasite from laying in their nest (Hobson and Sealy 1989). These behaviours may prevent brood parasites from accessing nests. Here, I test the hypothesis that variations in nest defence behaviours among potential host species towards brood parasites at the nest may limit host diversity in some species of brood parasites. I investigated the role of nest defence as a constraint to host diversity by comparing the nest defence behaviour of the grey warbler *Gerygone igata*, the primary host of the obligate brood-parasitic shining cuckoo *Chrysococcyx lucidus lucidus* on the main islands in New Zealand, to two other available and potentially suitable but unused species: South Island fantail *Rhipidura fuliginosa fuliginosa* and silvereye *Zosterops lateralis*. Grey warblers were as aggressive as South Island fantails and silvereyes and thus the hypothesis that nest defence behaviours limit host diversity in avian brood parasites was not supported.

## **2.2 Introduction**

Why some avian brood parasites use a limited diversity of host species, whereas others adopt a more generalist strategy is not well understood (Rothstein et al. 2002; Briskie 2003; Mermoz and Fernandez 2003; De Mársico and Reboreda 2008). One possible explanation for the low frequency of parasitism on potentially high-quality hosts is that the hosts possess effective anti-parasite defences (Scott 1977; Sealy and Neudorf 1995; Peer and Bollinger 1997; Davis et al. 2002; but see Peer et al. 2000). The most common forms of anti-parasite defences are aggressive prevention of parasites from accessing nests (Moksnes et al. 1990; Briskie et al. 1992; Røskaft et al. 2002; Gill et al. 2008; Feeney et al. 2012) and rejection of parasitic eggs through ejection (Sealy and Neudorf 1995; Moskát et al. 2002), burial (Sealy 1995; Guigueno and Sealy 2009, 2010), or nest desertion (Goguen and Mathews 1996; Hosoi and Rothstein 2000; Guigueno and Sealy 2009). Rejection of parasite nestlings also occurs but is less common (Langmore et al. 2003; Sato et al. 2010; Tokue and Ueda 2010; Delhey et al. 2011).

The presence of well-developed nest defence behaviours, such as striking, alarm calling, and nest-protection behaviour (i.e., sitting on the nest) in response to the threat of parasitism may prevent avian brood parasites from gaining access to their nests (Hobson and Sealy 1989; Moksnes et al. 1991; Sealy et al. 1998; Bártol et al. 2002; Røskaft et al. 2002; Welbergen and Davies 2008; Feeney et al. 2012; Latif et al. 2012). In some species, aggression by hosts may further deter brood parasites from approaching host nests due to the risk of injury (Molnár 1944; Davies and Brooke 1988; Feeney et al. 2012). Host defence may also cause brood parasites to avoid host nests for risk of alerting other potential host species in the vicinity to the presence of a

parasite, thus reducing the likelihood of successful parasitism in future attempts in the same area (Curio et al. 1978; Caro 2005; Davies and Welbergen 2009; Campobello and Sealy 2011a, 2011b). Lastly, some parasites may avoid aggressive hosts to prevent alerting predators and other brood parasites to the location of the nest (Smith et al. 1984; Krama and Krams 2005; but see Gill 1982; Gill et al. 1997a; Olendorf and Robinson 2000). Despite the benefits of deterring parasites from accessing the nest, nest defence behaviours are not without cost to the host.

Nest defence behaviours are not cost-free to hosts. They may attract predators (Martin et al. 2000) and brood parasites (Banks and Martin 2001), and may act as a nest location cue for intruders (Robertson and Norman 1977; Uyehara and Narins, 1995; Clotfelter 1998; Krams et al. 2007). By contrast, Gill et al. (1997a), found no significant intraspecific differences in the nest defense behaviours of parasitised and non-parasitised yellow warblers and red-winged blackbirds *Agelaius phoeniceus*. Hosts involved in nest defence behaviours also incur a cost to their own survival by reducing the time foraging and the extra expenditure in energy (Komdeur and Kats 1999) and risk injury or death when attacking the usually much larger parasite (Mclean 1987; Montgomerie and Weatherhead 1988; Sordahl 1990). In addition, the time spent on nest defence competes with the time required for parental care (Ueta 1999), which ultimately affects the survival of eggs and nestlings the hosts are attempting to protect.

Nest defence behaviours are expected when benefits outweigh costs. Risk assessment and the adaptive response based on the threat posed by the intruder, therefore, are important to the individual's survival and reproductive success (Curio et al. 1983; Caro 2005). In other words, hosts should only attack intruders that pose a risk to their eggs or nestlings but not intruders that pose little or no risk to their nest.

The ability of host species to specifically recognise an adult brood parasite has been studied extensively in hosts of the brown-headed cowbird *Molothrus ater* (Robertson and Norman 1977; Briskie and Sealy 1989; Burgham and Picman 1989; Hobson and Sealy 1989; Neudorf and Sealy 1992; Gill and Sealy 1996, 2004; Gill et al. 1997b; Sealy et al. 1998) and hosts of the common cuckoo *Cuculus canorus* (Smith and Hosking 1955; Moksnes and Røskft 1988, 1989; Moksnes et al. 1990; Duckworth 1991; Lindholm and Thomas 2000; Grim and Honza 2001; Bártol et al. 2002; Røskft et al. 2002; Honza et al. 2004; Hale and Briskie 2007; Welbergen and Davies 2008). Hosts respond to brood parasites, but not innocuous species, near their nests with mobbing, aggression and increased nest attentiveness (Hobson and Sealy 1989).

I investigated the role of nest defence behaviour as a constraint to host diversity by comparing nest defence of the grey warbler *Gerygone igata*, the primary host of the shining cuckoo *Chrysococcyx lucidus lucidus* on the main islands in New Zealand (Gill 1983a, Gill 1998), to two other available but unused species: South Island fantail *Rhipidura fuliginosa fuliginosa* (hereafter fantail) and silvereye *Zosterops lateralis*. Although there is experimental evidence of recognition of the shining cuckoo as a threat by the grey warbler (McLean and Rhodes 1991), evidence for other potentially suitable but non-used host species is only anecdotal (Michie 1948; Gill 1989; MacDonald and Gill 1991; Briskie 2007; pers. obs.). The specialist strategy of the shining cuckoo does not seem to have resulted from widespread egg rejection among alternative host species, based on the responses of a pool of species to artificial cuckoo eggs (Briskie 2003). However, rejection of parasitic nestlings has been demonstrated in Australia in two congeneric host species, the large-billed gerygone *Gerygone magnirostris* and mangrove gerygone *G. laevigaster* (Sato et al. 2010; Tokue and Ueda 2010). Thus, anti-parasite adaptations other than egg rejection

may have forced shining cuckoos to specialise on the grey warbler. The shining cuckoo competes with no other brood parasites in New Zealand (Gill 1998). The only other cuckoo native to New Zealand, the long-tailed cuckoo *Urodynamis taitensis*, is much larger and parasitises larger hosts. Ancestral and extinct populations may have played a part in the shining cuckoo's host specificity, but none are known (Gill 1998). In addition to being an avian brood parasite, the shining cuckoo depredates eggs and nestlings (Briskie 2007; pers. obs.).

In this chapter, I tested two predictions to assess the role of nest defence and nest attentiveness in host use by shining cuckoos:

### **Prediction #1**

If the intensity of nest defence among hosts is a factor in explaining variation in host diversity of brood parasites, grey warblers will be less aggressive toward shining cuckoos at their nests (the main species used as a host) than at either fantail or silvereve nests (unused but potential host species).

### **Prediction #2**

If nest attentiveness is a factor in host diversity for brood parasites, grey warblers will be less attentive to their nests than fantails or silvereys.

## **2.3 Methods**

### *Study site*

The study was conducted in Kowhai (42°23'S, 173°37'E) and Waimangarara Bushes (42°20' S, 173°40' E), which are located approximately 10 km from the town of Kaikoura on the South Island in New Zealand. The ecology of Kowhai Bush is described in detail in Gill (1980). Shining cuckoos are migratory and are present on

the study sites from late September to early February (Gill 1980; pers. obs.). Grey warblers, fantails and silvereyes are common and breed on these sites year-round (Briskie 2003). I studied the nest defence behaviour of hosts from 24 September to 16 December 2011 and 22 September to 11 December 2012. I also measured the responses at one silvereye nest on 10 November 2010. The periods of time I quantified nest defence of grey warblers, fantails and silvereyes corresponds with the period of peak breeding activity of each species (Ude Shankar 1977; Gill 1980; Gill et al. 1983; Higgins et al. 2006; Anderson et al. 2013). Shining cuckoos have the highest song intensity during this period, which coincides with peak grey warbler breeding activity (Gill 1980).

### *Study species*

I experimentally tested the response of grey warblers, fantails, and silvereyes to the presence of a shining cuckoo at the nest. The shining cuckoo ranges across Australasia and the South Pacific (Heather and Robertson 1996). The nominate race of the shining cuckoo, *Ch. l. lucidus* (Payne 2005), is an extreme host specialist, having been found to parasitise the grey warbler exclusively on the North, South and Stewart Islands (Gill 1998) despite the availability of alternative host species (Briskie 2003). In March, the shining cuckoo migrates north to the Bismark Archipelago and Solomon Islands where it resides until September (Gill 1983b) but some individuals may winter in the northern parts of the North Island (Robertson and Heather 2001). Fantails and silvereyes are potentially suitable as hosts but are currently not used by shining cuckoos (Gill 1998; Briskie 2003). Grey warblers, fantails, and silvereyes are common on the study sites (Briskie 2003), have overlapping breeding seasons (i.e., early October to late December)(Ude Shankar 1977; Gill 1980; Gill et al. 1983; Gill



1998; Higgins et al. 2006; Anderson et al. 2013), have similar body sizes and nestling diets (Higgins and Peter 2002; Higgins et al. 2006), and reject eggs of the shining cuckoo infrequently (Briskie 2003). However, these species are parasitised at different frequencies by the shining cuckoo and the lengths of their laying, incubation and nestling periods differ (Higgins and Peter 2002; Higgins et al., 2006). At Kowhai Bush, the natural frequency of parasitism on the grey warbler has been reported at 55% ( $n = 40$ ; Gill 1980) and 59% ( $n = 39$ ; Briskie 2003) of nests.

Grey warblers and fantails appear to have incubation periods of sufficient length (17-21 days and 13-16 days, respectively; Heather and Robertson 1996) to incubate the eggs of shining cuckoos (13-17 days, mean = 15.5 days,  $n = 3$ , Gill 1980; mean = 14.8 days,  $n = 3$ ; Gill 1998). In addition, fantails successfully hatched a freshly laid shining cuckoo egg that was experimentally inserted into their nest during the laying stage (Appendix 1). However, the incubation period of silvereyes, 11-12 days (Higgins et al. 2006), may be too short to successfully hatch a shining cuckoo egg or is likely to cause it to hatch later than the silvereye eggs, which puts it at a disadvantage to compete with the older and thus larger silvereye nestlings (Briskie 2007; Moskát and Hauber 2010). Both sexes incubate in fantails and silvereyes but only the female incubates in grey warblers (Ude Shankar 1977; Gill 1980; Heather and Robertson 1996; Higgins and Peter 2002; Higgins et al. 2006).

### *Experimental protocol*

I tested host nest defence by presenting models of a female shining cuckoo and female chaffinch *Fringilla coelebs* sequentially and in random order to nests of the grey warbler and two alternative but unused host species: silvereye and fantail as suggested in Sealy et al. (1998). I used three female shining cuckoo and two female chaffinch models to avoid pseudoreplication in sampling design (Hurlbert 1984; Sealy et al.,

1998). All models were in breeding plumage. The models were prepared from dead and frozen specimens. I prepared the models by first removing the intestines and the stomach and I filled the cavity that was formed with cotton. After the incision was sewn shut, the models were pinned to a wooden board in perching position and freeze-dried for ~ 24 hours. I used narrow green wires to attach the models' feet to branches near the nest during model presentations. Models were presented at random to eliminate order effects (Kamil 1988). The model used in each trial was chosen by flipping a coin: once to select the species and a second time to choose the specific model.

The chaffinch was included as a control treatment to determine whether potential host species possess a generalised response to all types of intruders at the nest or whether they possess specific adaptive responses depending on the threat, if any, posed by the intruder (Briskie et al. 1992; Sealy et al. 1998; Gill and Sealy 2004; Welbergen and Davies 2008). I chose the chaffinch because it does not threaten the reproductive success of the species tested, it is common on the study sites (Briskie 2003) and, because grey warblers, fantails, and silvereyes are familiar with it and have had the opportunity to assess the threat it poses (e.g., Mark and Stutchbury 1994; Grim 2005). The chaffinch differs from the shining cuckoo in colour, bill shape and tail length, which minimises the possibility that the hosts will misidentify the control (i.e., chaffinch) as a shining cuckoo (Grim 2005). The chaffinch (15 cm, 22 g) is approximately the same size as the shining cuckoo (16 cm, 25 g) (Robertson and Heather 2001).

I presented models to hosts one or two days after clutch completion (Hale and Briskie 2007). Although I controlled for nest stage, Grim (2005) did not find any relationship between nest stage and anti-cuckoo aggression in blackcaps *Sylvia*

*atricapilla* when faced with a common cuckoo. Alternatively, other studies have found nest defence response is stronger during the laying period when hosts are more vulnerable to parasitism than during the incubation stage when it might be too late in the nesting cycle for the parasite's egg to hatch (Hobson and Sealy 1989; Gill and Sealy 1996; Moskát 2005; Campobello and Sealy 2010). Grey warblers, fantails, and silvereyes may have responded more aggressively towards the shining cuckoo if they had been tested during the laying stage, when they are more vulnerable to successful parasitism, instead of early in the incubation stage. However, variations in response throughout the breeding cycle are unlikely because the shining cuckoo is a brood parasite and a nest predator and therefore, it may be adaptive for hosts to recognise the shining cuckoo as a threat throughout the nesting cycle (Grim 2005; Briskie 2007).

I placed the models 1 m from the nest and at the same height as the nest. Models were positioned to face the nest. I minimised the influence of host responses to models on the behaviour of other individuals by not testing nests within 50 m of other nests that were used for host defence tests because hosts may be influenced by their neighbours' behaviour (Gill and Sealy 2004; Davies and Welbergen 2009; Campobello and Sealy 2011a, 2011b). Each trial lasted for an average of  $4.91 \pm 0.30$  mins ( $n = 52$ , range: 3.38–5.00 mins) and began when one of the nest owners returned to  $< 5$  m of the nest. Some trials were shorter than 5 min because the host returned before I started the camera. In these cases, the time they returned to the nest was recorded and only the portion of video recording that fell within the first 5-min period after their return was analysed. Because total observation times varied among nests and observation periods, all responses were standardised by dividing them by the total time of the observation period, thus all scores are presented as rates or proportions.

To avoid habituation and carry-over effects, I conducted trials at each nest on subsequent days, except at one silvereye nest that I tested in 2010 where I waited only 20 min between trials (Sealy et al. 1998). All trials started between 6:20:56 AM NZST and 12:13:42 PM ( $202.54 \pm 11.65$  mins after sunrise,  $n = 52$ , range = 19 mins to 5 h 54 mins after sunrise), which coincides with the time shining cuckoos lay their eggs (Brooker et al. 1988; Briskie 2007). I video recorded all trials for later analysis.

I scored host nest defence behaviour using an assessment method based on De Marsico and Reboresda's (2008) method. Host nest defence behaviour was measured using the following criteria: attacks/min, proportion of time  $< 1$  m of model, proportion of time on the nest, songs/min, alarm calls/min, and time to first return to  $< 5$  m of nest. Attacks included strikes, when the host hit the model with its bill, usually after a quick short flight directed at the model, and close passes, which, as in strikes, involve rapid and directed flights to and past the model but without contacting the model. The time hosts incubated was not included in the proportion of time they spent  $< 1$  m from the model. Incubating birds were considered to be just beyond 1 m from the model.

The songs and alarm calls of the host species are defined in the sonograms in Figure 1. In grey warblers, only males perform songs (Gill 1980). However, females and independent juveniles perform subsongs (Gill 1983c). The song is described as a long series of trills or warbles that are given at a rate of approximately 8 notes/s typically lasting 5 s but up to 12 s (Higgins and Peter 2002; Fig. 1a of a shorter song). Other calls of the grey warbler lack proper quantitative descriptions in the literature. I defined an alarm call as a rapid succession of short warbles that lasted approximately 1 s (Fig. 1b). Several alarm calls were often given in succession. This definition loosely matches the description of the "twitter" call described in the literature, that is

a “ [s]hrill or rapidly repeated twitter...” (p. 326 in Higgins and Peter 2002). Observations suggest grey warblers give this call when angered, distressed or agitated (Higgins and Peter 2002). However, “twitters” have also been reported to be emitted by females when she has selected the nest site (Gill 1983c), in response to dropping a beetle, and in “faint, cheerful twittering conversation” between foraging birds (p. 326 in Higgins and Peter 2002). I also recorded a third call type that was not included in the analyses. It was given almost invariably with each close pass or strike. It sounded like a short whine or shriek. The call was repeated rapidly which made it difficult to quantify. It may be the same call that is described as “chatter” in the literature, which was emitted by grey warblers chasing each other in a territorial dispute (Gill 1980). However, it is not known if it was the chaser or the chased that emitted the “chatter” call (Gill 1980).

Male and female fantails sing but male songs are longer and more complex (Ude Shankar 1977) and are used to establish and maintain territories (Ude Shankar 1977). The fantail’s song has been described as being composed of a trill, a series of notes uttered in quick succession at the beginning, and a terminal part that is composed of three note phrases (Ude Shankar 1977)(Fig. 1c). Trills last from 0.5–1.5 s where several notes, varying in frequency, are uttered (Ude Shankar 1977). The terminal part is usually composed of 3–5 phrases but as few as one and as many as 19 phrases are uttered thus producing songs that range from 0.6 to 11 s long (Ude Shankar 1977). Alarm calls (also known as “Fast Type 1 calls”; Fig. 1d) are emitted by males and females when distressed and they are thought to alert other fantails in the area to a threat (Ude Shankar 1977). Alarm calls resemble contact calls but are emitted more rapidly, ~ 14 calls/15-s interval, as opposed to ~ 2 calls/15-s interval for contact calls (Ude Shankar 1977).

Silvereye calls are  $\sim 0.3$  s and within the range of 2–6 kHz (Robertson 1996; Potvin et al. 2011)(Fig. 1d). Variations within them differ among individuals and are not obvious to humans (Baker 2012). Silvereyes typically give  $\sim 5$  calls/ min, which have been described collectively as a song (Baker 2012). I counted calls individually instead of estimating the number of “songs” to more accurately measure vocalisations. Alarm calls (Fig. 1e) are produced at a rate of  $\sim 3$  alarm calls/s and involve frequencies, and multiple harmonics, from 2–8 kHz (J. L. Rasmussen pers. obs.).

In total, I recorded the responses of grey warblers at 14 nests, fantails at 11 nests, and silvereyes at 8 nests to a shining cuckoo and a chaffinch at the nest. However, only the responses at the nests of 10 grey warblers, 10 fantails, and 6 silvereyes were analysed. The responses of grey warblers at two nests were excluded because they did not return to  $< 5$  m of the nest within 30 min during the shining cuckoo trial only or for the shining cuckoo and chaffinch trials. The responses at two other grey warbler nests were excluded from the analyses because the trials took place before the return of the shining cuckoo to Kowhai Bush or because a loud tractor interrupted the chaffinch trial. The responses from fantails at one nest were excluded because the hosts were attacking another unidentified bird that led them away from the nest during one trial. Responses at two silvereye nests were not included because the hosts did not return  $< 5$  m of the nest within 30 min for the shining cuckoo trial or for the shining cuckoo trial and the chaffinch trial.

### *Statistical analyses*

The data were not normally distributed even after transformation (e.g., log transformation), therefore, nonparametric tests were used to analyse the data. I also performed a principal component analysis (PCA) on the data to reduce the number of

dependent variables. I performed a PCA on the following dependent variables: (1) time to return to  $< 5$  m of the nest, (2) attacks/min (strikes/min + close passes/min), (3) vocalisations/min (songs/min + alarm calls/min), (4) percentage of time  $< 1$  m of the model and, (5) percentage of time on the nest. I followed Kaiser's criterion (eigenvalues  $> 1$ ) in selecting principal components, which resulted in two principal components that together explained 68.32% of the total variance (Table 2.1). The proportion of time  $< 1$  m from the model, attacks/min, and vocalisations/min have high loading values for PC1, whereas time to return  $< 5$  m of the nest and the proportion of time spent on the nest have low and negative loading values for PC1. As the percentage of time  $< 1$  m of the model, attacks/min, and vocalisations/min represent aggressive behaviours, PC1 appears to represent overall aggressiveness. The loading values for the time to return  $< 5$  m of the nest are high for PC2, whereas all the other variables are negative thus PC2 appears to represent nest attentiveness. Grim (2005) hypothesised that latency reflects general incubation and nest-attentiveness patterns and is not related to nest defence behaviour. Wilcoxon matched-pairs signed rank tests were used to test the significance of component score differences between model types.

Kruskal-Wallis and Mann-Whitney U tests were used to test the significance of differences in response among and between species to the models, respectively. Four variables were chosen to compare nest defence at the nests among and between species: attack/min, percentage of time  $< 1$  m of the model, percentage of time spent on the nest, and time to return  $< 5$  m from the nest. Vocalisations were not used because it is not known whether shining cuckoos perceive the vocalisations of the three species in the same way. For example, it is not known whether shining cuckoos perceive the alarm calls of the grey warbler as more or less threatening than the calls

of fantails or silvereyes. All tests with  $p\text{-values} \leq 0.05$  were considered significant. All analyses were computed in STATISTICA 12 (Statsoft, Inc., Tulsa, OK, USA).

I did not test the significance of potentially confounding variables such as clutch size, time of season, time of day, number of previous visits to the nest, and nest concealment because these confounding variables were controlled by the within-subject design of the experiment (Kamil 1988). Also, other researchers have not found a relationship between the level of aggression of the host and some of these potentially confounding variables (Grim 2005; Campobello and Sealy 2010).

## **2.4 Results**

Grey warblers and fantails were significantly more aggressive (PC1) towards shining cuckoo models than chaffinch models (Table 2.2, 2.3). However, the level of aggression (PC1) shown by silvereyes did not differ significantly between model types (Table 2.2, 2.3). Nest attentiveness (PC2) did not differ significantly between model types for any of the species tested (Table 2.2, 2.3).

The differences among host species in the level of aggression towards a shining cuckoo model or a chaffinch model were not significant (Table 2.2, 2.4). On average, attacks/min on shining cuckoo models or chaffinch models did not vary significantly among or between host species (Table 2.2, 2.4). Likewise, the proportion of time that potential host species spent  $< 1$  m from the shining cuckoo model or chaffinch model did not vary significantly among or between species (Table 2.2, 2.4). However, the proportion of time each host species spent on the nest when confronted with a shining cuckoo or a chaffinch differed significantly among host species (Table 2.4). On average, fantails and silvereyes spent significantly more time on the nest than



grey warblers when confronted with a shining cuckoo model or a chaffinch model at the nest (Table 2.4). The time it took hosts to return to  $< 5$  m of the nest varied significantly among species regardless of the model type (Table 2.4). Grey warblers and silvereyes took significantly longer, on average, than fantails to return to the nest after a shining cuckoo model was setup at the nest. Grey warblers also took longer to return to  $< 5$  m the nest, on average, than fantails after a chaffinch model was set up at the nest. There was, however, no significant difference between the time to return to  $< 5$  m of the nest between grey warblers and silvereyes, as well as, between silvereyes and fantails.

## **2.5 Discussion**

The results suggest that host selection in the shining cuckoo is not constrained by variation in nest defence among potential host species. Grey warblers were equally as aggressive as fantails and silvereyes, two other species that are not currently used as hosts, to the presence of a shining cuckoo near their nest. However, the results suggest that it may be easier for shining cuckoos to access the nests of the grey warbler and silvereyes without being detected because grey warblers and silvereyes were less attentive. Grey warblers and silvereyes took longer than fantails to return to their nest after I installed a shining cuckoo model at their nest. The results also suggest that grey warblers and fantails recognised the shining cuckoo as a distinct threat when compared to a chaffinch, whereas silvereyes did not.

*Do grey warblers, fantails, and silvereyes recognise the shining cuckoo as a threat to their reproductive success?*

Sealy et al. (1998) suggested specific enemy recognition can serve as evidence of host-parasite co-evolution. Grey warblers and fantails responded more aggressively towards the shining cuckoo model than the chaffinch model, suggesting they recognise the shining cuckoo as a threat to their reproductive success, whereas they recognised that the chaffinch is not. The results support the hypothesis that there has been a coevolutionary interaction between both grey warblers and fantails with the shining cuckoo, but the nature of the relationship driving the coevolution (i.e., predator-prey or host-parasite) remains uncertain because I was unable to test the responses of the three species to the presence of a predator similar in size to a shining cuckoo at the nest. By contrast, silveryeyes responded similarly to the shining cuckoo and chaffinch. It is possible that silveryeyes have adopted a strategy of aggression towards any bird that approaches the nest, as seen in the eastern kingbird *Tyrannus tyrannus* (Bazin and Sealy 1993).

The finding that grey warblers recognized the shining cuckoo as a threat corroborates the findings of previous shining cuckoo model presentations. McLean and Rhodes (1991) found that females delayed their return to the nest when a shining cuckoo model was present at the nest but did not delay their return when a greenfinch was present. McLean and Rhodes (1991) also found that the shining cuckoo model was only attacked when the male returned with the female to the nest. Males emitted alarm calls and swooped at the model. By contrast, I did not find any difference between the shining cuckoo and the chaffinch models in the time it took grey warblers to return to < 5 m of the nest. This difference may be due to differences in methodology in determining when a bird “returned to the nest” between the studies. I did not record the sex of the birds at the nest because none were colour banded. It is possible that I recorded much higher aggressive behaviours towards shining cuckoos

because I left models at the nest for 5 min whereas McLean and Rhodes (1991) left the models at the nest for 1 min. Leaving the models at the nest for a longer period of time may have increased the probability that the male would return to the nest area and attack the model or it may have elicited more aggression from the hosts because the hosts may have perceived the intruder as more persistent.

*Does the intensity of nest defence behaviour differ between used and unused host species of the shining cuckoo?*

Differences in aggressiveness towards shining cuckoos among host species and potentially suitable but non-used hosts do not appear to limit shining cuckoos to using the grey warbler as their only host in New Zealand. If aggressiveness towards shining cuckoos limited host use, I would expect potentially suitable hosts to be significantly more aggressive towards shining cuckoos than grey warblers. Instead, the results indicate that grey warblers are equally as aggressive as two unused hosts (i.e., silvereyes and fantails) towards shining cuckoos. Therefore, differences in aggressiveness among hosts are likely not a contributing factor to host specialisation in the shining cuckoo in New Zealand.

In addition to being a brood parasite, the shining cuckoo is a predator of eggs and nestlings and thus may have a greater negative impact on the reproductive success of hosts than other nest predators and, in turn, place disproportionately greater selective pressure on host nest defences (Rothstein 1990; Barabás et al. 2004). Following a depredation event, nest owners lose all of their nestlings but are free to renest if it is not too late in the season. By contrast, following a successful cuckoo parasitism event, nest owners also lose all of their nestlings, but they are forced to care for the parasite for a prolonged period of time, which delays the start of their next

breeding attempt (Požgayová et al. 2009). Shining cuckoos are known to depredate the nests of all three species on the study site (Briskie 2007; J. L. Rasmussen pers. obs.) but only parasitise the grey warbler. Therefore, grey warblers might be expected to defend their nests more aggressively than fantails and silvereyes against shining cuckoos because cuckoos impose a higher reproductive cost on grey warblers. However, there was no significant difference among the species tested and this hypothesis is not supported.

The intensity of aggressiveness towards a species may be correlated with the threat posed by that species (e.g., Briskie et al. 1992; Gill and Sealy 2004; Welbergen and Davies 2008; Kleindorfer et al. 2013). Studies on adult brood parasite recognition have generally shown that appropriate hosts and hosts that have probably had a coevolutionary interaction with a brood parasite were more likely to be aggressive towards the parasite than species that were not appropriate hosts (Grim 2005). For example, superb fairy-wrens *Malurus cyaneus* had the strongest response for the calls of the species that posed the greatest risk of parasitism (Kleindorfer et al. 2013). Therefore, grey warblers may be more likely to defend their nests more aggressively than fantails and silvereyes against shining cuckoos because the cuckoos currently impose a higher reproductive cost on grey warblers, assuming the level of predation by shining cuckoos is the same across all three species. The trends in my data support this and show that grey warblers attacked shining cuckoo models more intensely than fantails or silvereyes. However, this difference in aggressiveness among the species was not significant. The non-significance of the result may simply be due to the small samples sizes, although the sample sizes I obtained produced significant differences in nest defence behaviours between model types in other hosts (Robertson and Norman 1976, 1977; Knight and Temple 1986; McLean and Maloney 1998). Even if larger

sample sizes produce significant results that suggest grey warblers are more aggressive than the two other unused hosts, which is possible given the trends in my data, my conclusion on the effect of aggressive nest defence on host selection in the shining cuckoo would remain the same.

The aggressive nest defence observed in fantails towards the shining cuckoo model can be explained by the fantail recognising the shining cuckoo as a nest predator. However, another plausible explanation for the aggressive behaviour of the fantail towards the cuckoo is that the aggressive nest defence behaviour may be a relic behaviour from the past when fantails were parasitised (Požgayová et al. 2009). Relic behaviours may be retained if their retention involves no cost to the individual (Rothstein 2001). Rothstein (2001) suggested this scenario for the presence of egg discrimination in species that are not currently parasitised. Požgayová et al. (2009) suggest the same for nest defence behaviour based on their finding that blackcaps defend their nests more aggressively when faced with an adult common cuckoo model than when presented with a nest predator, the jay *Garrulus glandarius*, or an innocuous species, the turtle dove *Streptopelia turtur*, despite the current lack of parasitism. Alternatively, Hale and Briskie (2007) found that song thrush *Turdus philomelos*, European blackbirds *T. merula*, and chaffinches introduced to New Zealand, and thus separated from the brood parasite in their native range, the common cuckoo, ~ 130 years ago lost their recognition of the common cuckoo but retained their ability to discriminate against and reject the eggs of common cuckoos. However, unlike song thrush, European blackbirds, and chaffinches, but like blackcaps, fantails have not been separated from the brood parasites present in their native range and thus a high level of aggression in nest defence may have been selected and maintained by

the cost of nest predation by brood parasites and not because of the costs of brood parasitism.

*Are grey warblers less attentive to their nests than fantails or silvereyes?*

Grey warblers and silvereyes took longer to return to their nests after placing a shining cuckoo model near their nests than fantails and thus the probability of detection for a shining cuckoo is probably lower when visiting a grey warbler or silvereye nest. Avoiding detection may be beneficial to the shining cuckoo to avoid being injured in an attack. Corroborating our findings, Briskie (2007) reported that shining cuckoos were undetected in two out of four direct observations of parasitism events on grey warbler nests. Greater nest attentiveness seen in fantails likely increases the probability that shining cuckoos will be detected when trying to parasitise nests of this species but there are no direct observations of shining cuckoos visiting the nests of fantails and silvereyes to support this hypothesis.

*Future directions*

It is not known whether grey warblers, fantails, and silvereyes would respond differently to the models if they were closer or farther from the nest. Experiments testing the effect of the distance of the model from the nest are necessary to get a better understanding of nest defence behaviour. Getting close to nests without being attacked may be advantageous for shining cuckoos because by being in close proximity to the nest they may be able to observe the nest to determine the best time to parasitise it (i.e., when the adults are gone). Shining cuckoo may be able to get closer to the nests of grey warblers without being attacked than the nests of potential but non-used host species. Unused hosts may not tolerate a shining cuckoo anywhere

near the nest, thus thwarting any opportunities for the cuckoo to observe the nest and determine the best time to parasitise it without being detected and attacked. Likewise, the probability of parasitism of great reed warblers *Acrocephalus arundinaceus* nests is correlated to their proximity to a common cuckoo vantage point such as a large tree or a telephone or power wire (Moskát and Honza 2000). However, adequate vantage points for shining cuckoos may be much closer to nests because grey warblers and other potentially suitable hosts nest in thicker and taller vegetation than great reed warblers, which nest in marshes.

Not all nest owners attacked the shining cuckoo model or emitted alarm calls after returning to < 5 m of the nest. The finding that some nest owners of all three species did not attack the shining cuckoo model or emit any alarm calls when the shining cuckoo model was present suggests: (1) there may be two types of individuals in the population (i.e., aggressive and tolerant), (2) prior experience with a shining cuckoo may be required for individuals to learn to recognise shining cuckoos as a threat to their reproductive success and to respond adaptively, or (3) aggressive behaviour may be conditional on the perceived threat of parasitism during a given breeding attempt. Future research is required to understand the variability of nest defence intensity among individuals within species, especially in the native species in New Zealand.

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Table 2.1. Loadings for variables included in principle component analysis. PC1 and PC2 explain 68.32% of the total variance.

Variable	Loadings	
	PC1	PC2
Attacks/min	0.60	-0.35
Vocalisations/min	0.74	-0.23
% time < 1 m of model	0.90	-0.13
Time to return < 5 m of nest	0.21	0.89
% time on nest	-0.70	-0.45



Table 2.2. Responses (mean  $\pm$  SE) of grey warbler, fantail and silvereye nest owners to chaffinch (control) and shining cuckoo models at nests.

Response	Species	n	Shining cuckoo		Chaffinch	
Time to return < 5 m from nest (min)	Grey warbler	10	6.83	$\pm$ 1.62	5.58	$\pm$ 1.46
	Fantail	10	0.70	$\pm$ 0.14	0.94	$\pm$ 0.41
	Silvereye	5	3.08	$\pm$ 1.25	4.28	$\pm$ 1.94
Strike/min	Grey warbler	10	6.17	$\pm$ 4.30	0.08	$\pm$ 0.08
	Fantail	10	1.12	$\pm$ 0.85	0.02	$\pm$ 0.02
	Silvereye	6	1.27	$\pm$ 1.27	0.00	$\pm$ 0.00
Close pass/min	Grey warbler	10	1.78	$\pm$ 0.74	0.74	$\pm$ 0.74
	Fantail	10	1.12	$\pm$ 0.54	0.50	$\pm$ 0.38
	Silvereye	6	0.13	$\pm$ 0.10	0.00	$\pm$ 0.00
Attacks/min (Strike/min + Close pass/min)	Grey warbler	10	7.95	$\pm$ 4.61	0.82	$\pm$ 0.82
	Fantail	10	2.22	$\pm$ 1.34	0.52	$\pm$ 0.40
	Silvereye	6	1.40	$\pm$ 1.36	0.00	$\pm$ 0.00
Songs/min	Grey warbler	10	2.59	$\pm$ 0.62	0.80	$\pm$ 0.42
	Fantail	10	0.76	$\pm$ 0.33	0.20	$\pm$ 0.09
	Silvereye	6	10.20	$\pm$ 9.37	5.20	$\pm$ 4.09
Alarm calls/min	Grey warbler	10	14.43	$\pm$ 5.42	0.14	$\pm$ 0.14
	Fantail	10	17.30	$\pm$ 9.59	1.66	$\pm$ 1.01
	Silvereye	6	2.63	$\pm$ 1.27	0.27	$\pm$ 0.27
Vocalisations/min (Songs/min + Alarm calls/min)	Grey warbler	10	17.03	$\pm$ 5.51	0.94	$\pm$ 0.42
	Fantail	10	18.06	$\pm$ 9.46	1.86	$\pm$ 0.99
	Silvereye	6	12.83	$\pm$ 10.34	5.47	$\pm$ 4.35
% time < 1 m from model	Grey warbler	10	43.78	$\pm$ 12.62	13.98	$\pm$ 7.52
	Fantail	10	32.88	$\pm$ 12.40	9.52	$\pm$ 5.03
	Silvereye	6	49.50	$\pm$ 21.26	8.17	$\pm$ 3.53
% time on nest	Grey warbler	10	0.00	$\pm$ 0.00	13.80	$\pm$ 10.14
	Fantail	10	43.65	$\pm$ 10.97	70.19	$\pm$ 12.37
	Silvereye	6	46.14	$\pm$ 19.64	72.72	$\pm$ 15.25

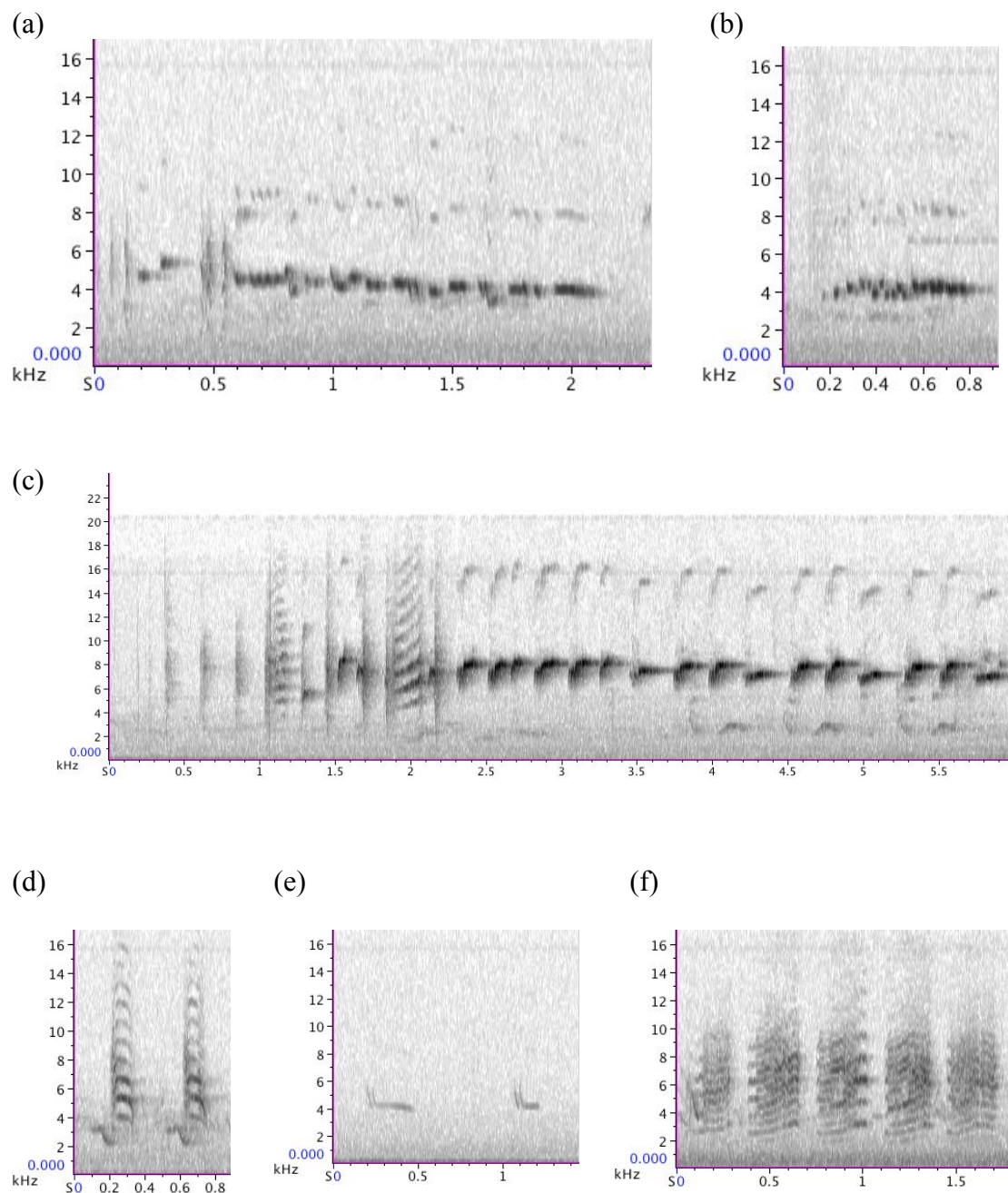
Table 2.3. PCA score summary (mean  $\pm$  SE) and values of statistical significance for behavioural differences between model types for grey warblers, fantails and silvereyes. Significant differences are indicated in bold.

Host species	Variable	n	Mean $\pm$ SE						Wilcoxon Matched Pairs Test	
			Shining cuckoo			Chaffinch			Z	P-value
Grey warbler	PC1	10	1.35	$\pm$	0.51	-0.18	$\pm$	0.19	2.19	<b>0.03</b>
	PC2	10	0.61	$\pm$	0.44	0.85	$\pm$	0.35	0.25	0.80
Fantail	PC1	10	0.19	$\pm$	0.55	-1.03	$\pm$	0.22	2.40	<b>0.02</b>
	PC2	10	-0.73	$\pm$	0.16	-0.59	$\pm$	0.13	0.25	0.80
Silvereye	PC1	6	0.34	$\pm$	0.79	-0.91	$\pm$	0.24	1.36	0.17
	PC2	6	-0.40	$\pm$	0.34	-0.17	$\pm$	0.36	0.94	0.35

Table 2.4. Differences among host species in nest defence behaviour in response to a shining cuckoo model or a chaffinch model at the nest. Significant differences are indicated in bold.

Variable	Model type	Host species						Kruskal-Wallis ANOVA		Mann-Whitney U test					
		Grey warbler		Fantail		Silvereye		H	p	Grey warbler vs. Fantail		Grey warbler vs. Silvereye		Fantail vs. Silvereye	
										Z	p	Z	p	Z	p
Attacks/ min	Shining cuckoo	7.95	± 4.61	2.22	± 1.34	1.40	± 1.36	1.98	0.371	-1.09	0.276	1.10	0.271	0.31	0.755
	Chaffinch	0.82	± 0.82	0.52	± 0.40	0.00	± 0.00	2.50	0.287	0.87	0.387	-0.65	0.519	1.35	0.176
% time < 1 m of model	Shining cuckoo	43.78	± 12.62	32.88	± 12.40	49.50	± 21.26	1.08	0.583	-0.99	0.322	-0.05	0.957	-0.61	0.540
	Chaffinch	13.98	± 7.52	9.52	± 5.03	8.17	± 3.53	0.54	0.762	-0.61	0.545	0.17	0.867	-0.52	0.602
% of time on nest	Shining cuckoo	0.00	± 0.00	43.65	± 10.97	46.14	± 19.64	11.88	<b>0.003</b>	3.37	<b>0.002</b>	-2.78	<b>0.005</b>	0.05	1.000
	Chaffinch	13.80	± 10.14	70.19	± 12.37	72.72	± 15.25	8.15	<b>0.017</b>	2.62	<b>0.009</b>	2.21	<b>0.027</b>	0.16	0.871
Time to return (min)	Shining cuckoo	6.83	± 1.62	0.70	± 0.14	3.08	± 1.25	10.30	<b>0.006</b>	-2.84	<b>0.005</b>	-1.16	0.245	-2.15	<b>0.032</b>
	Chaffinch	5.58	± 1.46	0.94	± 0.41	4.28	± 1.94	10.03	<b>0.006</b>	-3.14	<b>0.002</b>	-0.67	0.500	1.54	0.124

Figure 2.1. Sonograms of calls recorded at grey warbler, fantail and silvereye nests during model presentations: (a) grey warbler song, (b) grey warbler alarm call, (c) fantail song, (d) fantail alarm calls, (e) silvereye song and (f) silvereye alarm call. The x-axis represents time (s).



### **Chapter 3: Do red-winged blackbirds *Agelaius phoeniceus* respond to brown-headed cowbird *Molothrus ater* eggs with changes in incubation behaviour?**

#### ***3.1 Abstract***

Obligate brood parasites rely on other species, the hosts, to incubate their eggs and raise their offspring, which often reduces the hosts' reproductive output. This cost has led to the evolution of anti-parasite adaptations among hosts, which in turn, has led to better trickery by parasites. One of the most widespread anti-parasite adaptations is recognition and rejection of parasitism at the egg stage. Some hosts recognise they have been parasitised and desert their nests, eject parasitic eggs, or bury parasitised clutches. Yet, recognition of parasitism, determined by changes in incubation behaviour, without subsequent rejection has been demonstrated in some hosts that reject the eggs of parasites at intermediate frequencies. It is not known whether hosts that almost invariably accept the eggs of parasites recognise they have been parasitised. Here, the response, in terms of changes in incubation behaviour, of red-winged blackbirds *Agelaius phoeniceus*, an acceptor, and gray catbirds *Dumetella carolinensis*, a rejecter, to the presence of a brown-headed cowbird *Molothrus ater* egg in their nests was tested. Gray catbirds, but not red-winged blackbirds, spent more time at the nest as well as more time probing and probed and peered more frequently

when at the nest when it was parasitised then when it was not. The percentage of time spent peering did not change when the clutch was parasitised for either species. The findings suggest that red-winged blackbirds, a species that accepts the eggs of cowbirds, do not recognise they have been parasitised.

### ***3.2 Introduction***

Obligate avian brood parasites rely solely on other species to incubate their eggs and raise their young. Raising a brood parasite involves a cost to the host in terms of time, energy, and reproductive output (Øien et al. 1998; Lorenzana and Sealy 1999; Davies 2000). This cost has acted as a selective pressure on hosts to evolve counter-adaptations that reduce or eliminate the cost of parasitism (Rothstein 1990; Davies 2000). In turn, parasites have evolved adaptations to further outwit hosts and continue to parasitise them successfully (Rothstein 1990; Davies 2000). The antagonistic and reciprocal interaction between hosts and parasites is believed to either continue in perpetuity or until an evolutionary impasse is reached (Janzen 1980; Futuyma 1998). This co-evolutionary interaction has been termed an “evolutionary arms-race” (Dawkins and Krebs 1979).

The evolutionary arms race between avian brood parasites and their hosts has led to host defences against parasitism such as nest desertion (Goguen and Mathews 1996; Hosoi and Rothstein 2000), clutch burial (Sealy 1995, Guigueno and Sealy 2009), as well as egg (Rothstein 1975, 1982) and nestling rejection (Langmore et al. 2003; Sato et al. 2010; Tokue and Ueda 2010; Delhey et al. 2011). The recognition of parasitism by many hosts and potential hosts has been experimentally demonstrated by the rejection of parasitised clutches and the ejection of parasitic eggs. These anti-

parasite behaviours suggest hosts possess the cognitive abilities required to recognise that they have been parasitised and the physical capabilities for rejection in the case of egg and nestling ejection. However, overt rejection of parasitism may not be the only endpoint after recognition because some individuals within species have been shown to recognise the eggs of parasites without rejecting them, suggesting that rejection may be a conditional response (Hauber and Sherman 2001; Moskát and Hauber 2007; Antonov et al. 2009; Guigueno and Sealy 2012; Soler et al. 2012). Furthermore, rapid changes in rejection frequency in some host populations (Brooke et al. 1998), changes in response from individuals that were repeatedly experimentally parasitised (Soler et al. 2000; Honza et al. 2007; but see Vikan et al. 2009; Peer and Rothstein 2010), and the demonstration that some hosts recognise they have been parasitised or recognise parasitic eggs but do not reject (Antonov et al. 2009; Guigueno and Sealy 2012; Soler et al. 2012) also suggest there is a conditional component to rejection behaviour (Hauber and Sherman 2001; Moskát and Hauber 2007).

Incubation behaviour has been defined as the movements and organisation of daily activities of birds when they have eggs (Thomson 1964) and changes in incubation behaviour likely indicate recognition of parasitism because the probability of rejection is correlated with the proportion of time a bird investigated its clutch in in yellow warblers *Setophaga petechia* and eastern olivaceous warblers *Iduna pallida* (Guigueno and Sealy 2012; Soler et al. 2012). Additionally, changes in incubation behaviour have also been demonstrated in response to threats at the nest (McLean and Rhodes 1991; Požgayová et al. 2009).

However, recognition of parasitism, in terms of changes in incubation behaviour, without rejection has been reported in yellow warblers, eastern olivaceous warblers, and rufous-tailed scrub robins *Cercotrichas galactotes*, three species that

reject the eggs of brood parasites at intermediate frequencies (Antonov et al. 2009; Guigueno and Sealy 2012; Soler et al. 2012). Yellow warblers, a species that rejects the brown-headed cowbird *Molothrus ater* (*hereafter* cowbird) eggs infrequently (0%, n = 16, Rothstein 1975 to 15%, n = 189, Sealy 1995) through burial or desertion, spent more time peering, shuffling, and probing their clutches after the experimental addition of cowbird egg, which suggests they recognise that they have been parasitised (Guigueno and Sealy 2012; but see Sealy and Lorenzana 1998). Nevertheless, not all yellow warblers that changed their behaviour in response to the presence of a cowbird egg in their nest later deserted or buried their clutches, which suggests recognition of parasitism may not necessarily lead to rejection (Guigueno and Sealy 2012). However, it is also possible that yellow warblers did not recognise the insertion of a cowbird egg as an act of parasitism and instead simply responded to the change in the appearance of their clutch (Guigueno and Sealy 2012).

The findings of two other studies investigating recognition in yellow warblers suggest they do not recognise cowbird eggs, but these studies measured different or fewer variables. At the egg stage, Sealy and Lorenzana (1998) concluded yellow warblers do not recognise their own eggs because the amount of time they peered into the nest before settling on the nest did not differ before versus after the addition of a cowbird egg. At the nestling stage, McMaster and Sealy (1999) did not find any difference in female attentiveness or rate of food delivery at control and experimentally parasitised nests in yellow warblers.

Eastern olivaceous warblers pecked more and incubated less when their clutches included a common cuckoo *Cuculus canorus* egg, but they did not spend more or less time looking at the eggs after a cuckoo egg was added to the clutch (Antonov et al. 2009). Despite the changes in behaviour in response to the presence of



a common cuckoo egg, there was no significant correlation between rates of egg pecking and rejection (Antonov et al. 2009). Likewise, rufous-tailed scrub robins rejected only 55% of artificial eggs they pecked and 20% of real house sparrow *Passer domesticus* eggs that they pecked (Soler et al. 2012). Rufous-tailed scrub robins only pecked foreign eggs, did not peck their own eggs after the ejection of a foreign egg, and did not peck any eggs at control nests, which suggests they actually recognised foreign eggs despite not always rejecting them (Soler et al. 2012).

Not all host species respond to the presence of a parasite's egg by changing their incubation behaviour. Reed warblers *Acrocephalus scirpaceus*, which reject the eggs of common cuckoos at intermediate frequencies, did not change their incubation behaviour in response to an artificial common cuckoo egg within 30 min of being parasitised experimentally even at nests where a model adult cuckoo was presented immediately preceding the insertion of the model egg (Honza et al. 2004).

Whether conditional acceptances of avian brood parasitism are widespread is not known nor is it understood how species that regularly accept the eggs of brood parasites respond to foreign eggs in their nests, in terms of changes in incubation behaviour (Antonov et al. 2009; Guigueno and Sealy 2012). The goal of the present study was to determine whether red-winged blackbirds *Aegelaius phoeniceus* recognise that they have been parasitised despite infrequent rejection (i.e., ejection or desertion) (Rothstein 1975; Ortega and Cruz 1988; Kren 1996; Clotfelter and Yasukawa 1999; Capper et al. 2012). However, red-winged blackbirds may have the perceptual abilities required to recognise cowbird eggs because they can distinguish between broken eggs, which they eject, and eggs from which a chick is hatching that were experimentally introduced into the clutch, which they do not eject (McMaster and Sealy 1998). The frequency at which broken eggs are ejected from the nest

decreases when the clutch starts to hatch, potentially to prevent ejecting eggshells containing hatchlings (McMaster and Sealy 1998).

I tested whether the presence of a cowbird egg in the nest would change the behaviour of incubating female red-winged blackbirds. I then compared the response of female red-winged blackbirds, in terms of changes in incubation behaviour, to the response of a species that typically rejects cowbird eggs, the gray catbird *Dumetella carolinensis*, to a cowbird egg in the nest. I measured and compared the incubation behaviour of red-winged blackbirds and gray catbirds before, during, and after an artificial cowbird egg was introduced to their clutches.

I predicted that red-winged blackbirds would respond to the addition of a cowbird egg to their nest with significant changes in incubation behaviour if they recognise a cowbird egg in their nest. I also predicted that if red-winged blackbirds recognised a cowbird egg in the nest, they would respond with changes in incubation behaviour similar to those of gray catbirds. This study improves our knowledge of how widespread recognition of the parasitic eggs is among host species that reject seldomly.

### **3.3 Methods**

#### *Study site*

This study was conducted in the marshes and adjacent forested areas in and near Delta, Manitoba, Canada (50°11'N, 98°19'W). The ecology of the area has been described in detail in Mackenzie (1982). Breeding red-winged blackbirds and gray catbirds are abundant in the marshes and adjacent forested regions, respectively, near

Delta during the spring and summer months (Rasmussen et al. 2010; Capper et al. 2012).

The rate of cowbird parasitism in red-winged blackbird nests near Delta varies among years and ranges from 2–35% per year (Weatherhead 1989; Neudorf and Sealy 1994; Grant and Sealy 2002; Woolfenden et al. 2004; Capper et al. 2012). This rate is very similar to the parasitism rates reported for other red-winged blackbird populations such as in Wisconsin where rates of parasitism were 2–32% of nests per year over a 14-year period (Clotfelter and Yasukawa 1999) but have been reported to be as low as 1.6% in northern Louisiana (Brown and Goertz 1978) and as high as 42–74% in Lincoln County, North Dakota (Blankespoor et al. 1982). Intraspecific brood parasitism has been reported to be less than 1% in red-winged blackbirds (Gibbs et al. 1990; Harms et al. 1991; Westneat 1992, 1993; Gray 1994). By contrast, the rate of parasitism is difficult to determine because gray catbirds remove cowbird eggs from their nests but Scott (1977) reported that 44% of gray catbird nests in a study in Ontario were parasitised. It is not known whether intraspecific parasitism occurs in catbirds.

### *Study species*

Red-winged blackbirds accept the eggs and raise cowbirds despite being physically able to eject them from the nest (Rothstein 1975; Ortega and Cruz 1988; Rasmussen et al. 2010). Red-winged blackbirds have been reported to grasp-eject objects of up to 20.1 mm in width and thus should be capable of grasp-ejecting a cowbird egg (Ortega et al. 1993). Red-winged blackbirds in two sites in southern Wisconsin were reported to desert parasitised nests more frequently, 39.2% (n = 143) and 31.6% (n = 79), than unparasitised nests, 8.4% (n = 203) and 5% (n = 858),

respectively (Clotfelter and Yasukawa 1999). Alternatively, near Delta, Manitoba, red-winged blackbirds accepted and ejected artificial cowbird eggs at 81 (91%) and 8 (9%) of 88 nests, respectively, and no nests were deserted (Capper et al. 2012).

The number of young that fledge from red-winged blackbird nests is usually lower in parasitised nests than non-parasitised nests, but not always (Lorenzana and Sealy 1999). The reduction in reproductive success of red-winged blackbirds caused by cowbirds is mostly due to the removal of a host egg when laying their own (Røskoft et al. 1990; Clotfelter and Yasukawa 1999) and this cost is irrecoverable (Sealy 1992; Lorenzana and Sealy 2001; Stokke et al. 2008). Clotfelter and Yasukawa (1999) found no negative effects on the reproductive success of red-winged blackbirds after the laying of a cowbird egg. They suggested that because of this lack of selection after parasitism there is likely less pressure for them to evolve egg discrimination and rejection. Instead, selection may favour red-winged blackbirds that behave in ways to prevent cowbirds accessing their nests, such as nest defence, especially because cowbirds are egg predators as well as brood parasites (Clotfelter and Yasukawa 1999). Only females incubate, typically beginning with the penultimate egg, but has been reported in some individuals to begin as early as the laying of the second egg and as late as 2 days after laying the last egg (Yasukawa and Searcy 1995).

In contrast to red winged blackbirds, gray catbirds eject cowbird eggs quickly (Rothstein 1975; Lorenzana and Sealy 2001; Rasmussen et al. 2009), typically by grasp-ejection (Rasmussen et al. 2009). Gray catbirds grasped-ejected real cowbird egg in 14 of 17 video recorded ejections of real cowbird eggs (Rasmussen et al. 2009). The other three eggs were ejected by puncture-ejection (Rasmussen et al. 2009). Ejection may have been selected in gray catbirds because raising a cowbird

costs gray catbirds more (0.79 gray catbird fledglings) than ejecting a cowbird egg (0.0022 fledglings per ejection) (Lorenzana and Sealy 2001). As in red-winged blackbirds, only female catbirds incubate (Smith et al. 2011), some beginning with the second egg and with full incubation beginning when the clutch is complete or nearly completed (Smith et al. 2011).

Cowbird eggs differ in shape, colour, and maculation patterns from the eggs of gray catbirds and red-winged blackbirds (Ortega and Cruz 1988; Lowther 1993; Yasukawa and Searcy 1995; Smith et al. 2011; pers. obs.) and, therefore, possibly provide a strong visual cue.

### *Experimental procedure*

I tested the effect of the presence of a cowbird egg in the nest on the behaviour of incubating female red-winged blackbirds and gray catbirds by video-recording incubating females. A within-subject design was used to control for individual differences in behaviour (Gravetter and Forzano 2003). Each nest was recorded on 3 subsequent days. I waited 24 h between recordings to minimise carry-over effects. On the first and third days, I recorded incubation behaviour at the nest without manipulating the clutch. These recordings served as control treatments (PRE and POST trials) for the experiment. On the second day, I recorded incubation behaviour immediately after I experimentally parasitised the nest with an artificial cowbird egg that was painted to appear like a cowbird egg to the clutch (CBE trial). To control for the change in clutch size and volume caused by the addition of a cowbird egg, I removed one host egg from nests for the duration of the CBE trial at 8 of the 23 red-winged blackbird nests where I inserted artificial cowbird eggs. I tested all gray catbird nests with artificial cowbird eggs and did not remove any gray catbird eggs

during the trials used in the analysis. Egg removal during parasitism with real cowbird eggs did not have a significant effect on incubation period, hatching order, or hatching spread in yellow warblers (McMaster and Sealy 1997). However, its effect on cowbird egg hatching success varied among years (McMaster and Sealy 1997).

Video cameras were set up ~ 5 m from the nest. Video cameras were set on tripods and covered with burlap cloth to blend with the surrounding vegetation. It is not known how the subjects perceived the camera apparatus. However, any effect the camera apparatus may have had on the subjects was controlled by using camera apparatuses with similar appearances at each nest and for each trial. I used Sony® DCR cameras with extended life batteries and internal hard drives for data storage to video-record nests. I quantified the behavioural data from all recordings to eliminate inter-observer variability.

From the video recordings, I measured six variables that likely indicate the host was responding to the experimental manipulation and that have been used in previous studies to measure behaviour at the nest (Sealy and Lorenzana 1998; Antonov et al. 2009; Guigueno and Sealy 2012). This included the proportion of time at least one red-winged blackbird or gray catbird was present at the nest, which I expected would be higher on the day the experimental cowbird egg was present in the nest because the host would spend more time investigating the clutch. I also measured the frequency by which birds peered at the eggs when at the nest, proportion of time peering at the eggs when at the nest, frequency of probing the eggs when at the nest, and proportion of time probing the eggs when at the nest. I expected would all increase on the day the parasitic egg was present because the host would spend more time investigating the new egg in the nest (Antonov et al. 2009; Guigueno and Sealy 2012; Soler et al. 2012).

Peering was recorded when a bird stood on the edge of the nest and looked at the eggs, as indicated by the lowering of the bird's head towards the nest cup and by the orientation of the bill down and towards the eggs. Probing has been defined previously as when a female lifts her body from a position where she is settled on eggs and then turns or rotates the eggs with her bill (Sealy and Lorenzana 1998; Deeming 2002; Guigueno and Sealy 2012). Probing behaviour includes pecking and tremble-thrusts (Deeming 2002; Underwood and Sealy 2011). Tremble-thrust is a motion used by some species to rotate their eggs during incubation, which typically involves vibrations of the body and placement of the head deep in the nest (Deeming 2002). I used probing to describe both tremble-thrusts and pecking because I could not differentiate between tremble-thrusts and pecking with certainty because the birds' heads were not always visible when these behaviours were performed (Underwood and Sealy 2011).

I video recorded 45 red-winged blackbird nests, but used the data from only 23 nests in my analyses. The video recordings taken at 12 of the 45 nests were excluded either because the nests were obscured by vegetation (5 of 12 nests), or because I was not able to record incubation behaviour in response to all three trials (i.e., PRE, CBE, and POST trials), due to depredation (3 of 12 nests), human disturbance (2 of 12 nests), desertion (1 of 12 nests), or rain (1 of 12 nests). I tested 23 of the 45 nests with artificial eggs. The other 10 nests were tested with real cowbird eggs. I present here only the data from the 23 nests I tested with artificial cowbird eggs, because this controlled for egg type when comparing the incubation behaviour of red-winged blackbirds to the incubation behaviour of gray catbirds, which were all tested with artificial cowbird eggs. The data from the 10 red-winged blackbird nests tested with real cowbird eggs are presented in Appendix 2 where I compare the effect of artificial

or real egg type on the incubation behaviour of red-winged blackbirds.

I video recorded incubation behaviour at red-winged blackbird nests in which I inserted an artificial egg from 21 May to 18 June 2012. In total, I analysed 203.02 h of incubation behaviour at 23 nests. For each trial I analysed an average of  $2.94 \pm 0.02$  h ( $n = 69$ , range: 1.83–3.00) of video. Trials began as early as 5.23 min before sunrise and as late as 120.40 min after sunrise (mean =  $41.65 \pm 3.33$  min,  $n = 69$ ) but the maximum difference among trial start times at individual nests averaged  $24.80 \pm 4.23$  min ( $n = 23$ , range: 2.72–81.32).

I video recorded gray catbird incubation behaviour from 20 May to 19 June 2012. I video recorded incubation behaviour at 19 gray catbird nests, but used the data from only nine nests. Ten nests were excluded from the analysis either because catbirds ejected the egg within 30 min of returning to the nest (7 of 10 nests), vegetation obscured the view of the nest (1 of 10), the nest was depredated between the first control trial and the cowbird egg trial (1 of 10), or because a catbird returned for less than 7 s during the 3 hour observation period of the first control trial (1 of 10). I analysed 74.24 h of incubation behaviour at 9 nests. Observation times were  $2.86 \pm 0.12$  h,  $2.39 \pm 0.25$  h, and  $3.00 \pm 0.00$  h for the PRE, CBE, and POST trials respectively. Some trials were stopped before the 3 hours of observation time if it started to rain or when a catbird ejected the cowbird egg. I attempted to begin all trials at sunrise because cowbirds lay their eggs shortly before sunrise (McMaster et al. 2004). Trials began as early as 3.9 min and as late as 118 min after sunrise ( $n = 27$ , mean =  $51.88 \pm 5.16$  min) but the maximum difference among trial start times at individual nests averaged  $34.19 \pm 5.32$  min ( $n = 9$ ; range: 10.58–52.87).



### *Model eggs*

Artificial eggs, similar in size, mass, and appearance to real cowbird eggs, were made of plaster of Paris and sanded, painted and polished to give them the appearance of real cowbird eggs. All models were painted with Folkart™ non-toxic acrylic paint. Color #940 (Coffee Bean) was used for the maculations and colour #901 (Whicker White) for ground colour. Reflectance measurements were taken for each artificial egg used in this experiment. Unfortunately, the measurements were erroneous because the spectrometer was not functioning properly at the time the measurements were taken. I will take the measurements again and present them in any published papers that may result from this work.

The mass of the artificial eggs was kept near the mass of real cowbird eggs by inserting pieces of polystyrene in their cores. Artificial eggs used in red-winged blackbird and gray catbird nests did not differ significantly in either mass ( $3.22 \pm 0.07$  g vs.  $3.31 \pm 0.11$  g, Welch  $t = 0.69$ , d.f. = 14,  $p = 0.50$ ), width ( $17.54 \pm 0.03$  mm vs.  $17.53 \pm 0.08$  mm,  $t = 0.12$ , d.f. = 10,  $p = 0.91$ ), or length ( $22.93 \pm 0.08$  mm vs.  $23.08 \pm 0.09$  mm,  $t = 1.23$ , d.f. = 22,  $p = 0.23$ ). Artificial eggs used in red-winged blackbird and gray catbird nests did not differ significantly from real cowbird eggs (data from Sealy 1992) in mass ( $3.24 \pm 0.06$  g vs.  $3.14 \pm 0.04$  g,  $t = 1.45$ , d.f. = 63,  $p = 0.15$ ), but were slightly wider ( $17.54 \pm 0.03$  mm vs.  $16.36 \pm 0.09$  mm,  $t = 12.44$ , d.f. = 91,  $p < 0.01$ ) and longer ( $22.97 \pm 0.07$  mm vs.  $21.07 \pm 0.12$  mm,  $t = 13.68$ , d.f. = 106,  $p < 0.01$ ) than real cowbird eggs.

### *Statistical analyses*

The data were not normally distributed even after transformation (i.e., log transformation for all data or arcsine transformation for proportions and square root

transformation for frequencies). I used a non-parametric statistical procedure, Friedman two-way analysis of variance (ANOVA) by ranks, which is the non-parametric equivalent of a repeated-measures ANOVA, to test the differences among treatments. This procedure tests for differences in the distribution of scores of three or more dependent samples, in this case the three trials (Daniel 1999). In general, the null hypothesis for this test is that all treatments, three in this study, all have identical effects (Daniel 1999). The alternative hypothesis for this test is that at least one treatment tends to yield larger observations than at least one of the other treatments (Daniel 1999). If the Friedman's test suggested a significant difference among the trials, I conducted Wilcoxon matched-pairs signed rank tests to test the significance of differences in incubation behaviour between each of the observation periods. Wilcoxon matched-pairs signed rank tests consider the magnitude and the direction of the difference in scores (Gravetter and Forzano, 2003). Each variable was analysed separately. All tests with  $p$ -values  $\leq 0.05$  were considered significant. All analyses were computed in STATISTICA 12 (Statsoft, Inc., Tulsa, OK, USA). Values are reported as means  $\pm$  SE.

### **3.4 Results**

#### *Effect of experimental parasitism on incubation behaviour*

The incubation behaviour of red-winged blackbirds did not differ significantly among trials except in probing frequency (Table 3.1). The difference in probing frequency was significantly lower during POST trials than during CBE trials (Wilcoxon matched pairs test:  $Z = 2.89$ ,  $p = < 0.01$ ), whereas the difference between the PRE and CBE trials ( $Z = 0.82$ ,  $p = 0.41$ ) or the PRE and POST trials ( $Z = 1.22$ ,  $p = 0.22$ ) were not

significant (Table 3.1). None of the 22 artificial eggs recovered from red-winged blackbird nests had visible peck marks. One artificial cowbird egg was lost after removing it from the nest.

The behaviour of gray catbirds differed significantly among trials for all measured incubation behaviour variables except for peering frequency and on-bout frequency (Table 3.1). Wilcoxon matched-pairs signed rank tests indicate that incubation behaviour variables that differed significantly among trials were significantly higher during CBE trial than the PRE or POST trials and that there was no significant difference between the PRE and POST trials (Table 3.2). In other words, gray catbirds acted differently when a cowbird egg was present in their nest than when there was no cowbird egg in their nest, which was expected because they eject cowbird eggs (Lorenzana and Sealy 2001).

Temporary removal of a host egg during the CBE trial did not affect the incubation behaviour of red-winged blackbirds. The behaviour of red-winged blackbirds was the same, in terms of significant differences between observation periods, at nests where an experimental cowbird egg was added without removing one of the host's eggs and at nests where an experimental egg was switched for one of the host's eggs (Table 3.3).

I video-recorded gray catbirds ejecting artificial cowbird eggs at 15 nests. In all cases, gray catbirds grasped the egg. The mean time from first arrival to the nest after experimental parasitism to ejection was  $68.92 \pm 18.93$  min (range: 0.033–237.22). Eight ejections were by individuals that were sitting on the clutch immediately before the ejection occurred, thus indicating that they were female. The other seven ejections were from individuals that arrived at the nest and immediately ejected the model without first sitting on the clutch, thus, I could not tell for sure if they were male or

female. Three other eggs were accepted for the duration of the video recordings (390.08, 414.8, and 433.9 min). Of the 3 artificial cowbird eggs that were recovered from gray catbird after trials, none had peck marks. Gray catbirds did not eject or damage any of their own eggs during any of the ejections.

### ***3.5 Discussion***

The results suggest red-winged blackbirds, a species that accepts cowbird eggs, apparently do not recognise cowbird eggs in their nests as a threat within three hours of parasitism, whereas gray catbirds recognise cowbird eggs as a threat within the first three hours of parasitism. Gray catbirds spent significantly more time at the nest, probed the eggs significantly more often, and spent significantly more time peering and probing when there was a cowbird egg in their nest than when there was not. By contrast, red-winged blackbirds did not change their incubation behaviour significantly in response to the presence of a cowbird egg in the nest, although they probed their eggs significantly less frequently in the POST trial than during CBE trial but no significant differences in probing frequency were found between any of the other trials. However, this pattern of behavioural change, in terms of significant differences between trials, was not the pattern expected of hosts that respond to the presence of a cowbird egg as a threat to their reproductive success in the nest as was seen in gray catbirds. If red-winged blackbirds perceived cowbird eggs, or simply a change to their clutch, as a threat, I expected a significant increase in probing frequency between the PRE and the CBE trials as was seen at gray catbird nests. Instead, red-winged blackbirds appear only to have responded by significantly

decreasing their probing after the removal of a cowbird egg from their nests. Despite the small response from red-winged blackbirds, it is possible that the significant decrease in probing frequency of red-winged blackbirds to changes in their clutch may be an early precursor to the recognition and rejection of parasitic eggs.

My negative result for the effect of the presence of a cowbird egg on the incubation behaviour of red-winged blackbirds corroborates with the findings of previous research on red-winged blackbirds (Clotfelter 1997). Clotfelter (1997) found no significant difference in the nest defence behaviour or nestling feeding frequency at parasitised and non-parasitised red-winged blackbird nests (Clotfelter 1997). Additionally, previous exposure to an adult model cowbird had no effect on nest defence behaviour and nestling feeding rate at parasitised and non-parasitised red-winged blackbird nests (Clotfelter 1997).

Soler et al. (2012) and Antonov et al. (2009) measured the pecking behaviour of eastern olivaceous warblers and rufous-tailed scrub wrens, respectively, and recorded significant differences in the amount of pecking after an experimental egg was added to a nest. I did not record pecking specifically because the camera could not be set up close enough to the nest and at an angle where I could see in the nest without disturbing the birds. However, the absence of peck marks on the artificial cowbird eggs in trials at red-winged blackbird nests suggests they were not pecked. Pecking by red-winged blackbirds would have likely left marks on the eggs because a much smaller species, eastern olivaceous warbler, left peck marks on artificial common cuckoo eggs, which were made of polymer clay, which has a similar hardness to plaster of paris (Antonov et al. 2009). Alternatively, gray catbirds may have not left a mark on the cowbird eggs because they may have pecked them lightly. Soler et al. (2012) found that rufous-tailed scrub robins pecked some experimental eggs with very

little force.

The change in behaviour in response to a parasite's egg by gray catbirds corroborates the findings in other species that recognise parasitic eggs. Like gray catbirds, yellow warblers probed their clutches more when parasitised (Guigueno and Sealy 2012). And, like gray catbirds, the amount of time eastern olivaceous warblers peered into the nest did not differ significantly between periods when their nest was parasitised or periods when it was not parasitised (Antonov et al. 2009). However, gray catbirds spent more time at the nest after it was parasitised than before it was parasitised, whereas there was no significant difference in the amount of time eastern olivaceous warblers spent at the nest before or after their nest was parasitised (Antonov et al. 2009).

Acceptance rates of red-winged blackbirds and gray catbirds were likely overestimated because of the short time I left experimental cowbird eggs in the nest and because of the artificiality of the experimental cowbird eggs (Antonov et al. 2009). The accepted standard among researchers for the time an egg must remain in an active nest before being considered accepted by the host is at least five days (Rothstein 1975). Instead, my results are based on the duration of one recording session lasting ~ 6 h. In total, 83% (15 of 18 eggs) of the experimental cowbird eggs were ejected by catbirds within 5 h. Lorenzana and Sealy (2001) found that 56% of the artificial eggs they inserted in gray catbird nests were ejected within 5 h ( $n = 90$ ) with 96% of them eventually being ejected after 2 or 3 days. The factors responsible for the difference in the rapidity of ejection are not known but may be attributable to differences in the appearance egg models used (Hale and Briskie 2007), variations in egg ejection behaviour among years (Brooke et al. 1998), or chance.

It was previously found that gray catbirds use both grasp-ejection and puncture-

ejection to eject cowbird eggs (Rasmussen et al. 2009). By contrast, gray catbirds grasp-ejected exclusively in this study, possibly because model eggs were used in the present study and real eggs were used by Rasmussen et al. (2009). The hardness of the artificial cowbird eggs may have precluded puncture-ejection and therefore, the rate of grasp-ejection versus puncture-ejection presented in the present study likely does not represent the true rate when faced with real cowbird eggs (Sealy and Neudorf 1995; Antonov et al. 2010). The hardness of the artificial eggs was believed to eliminate puncture-ejection as an option for rejection for the eastern olivaceous warbler, which likely deserted experimentally parasitised clutches instead (Antonov et al. 2009). However, like gray catbirds, warbling vireos are flexible in the method of ejection they use (Sealy 1996; Underwood and Sealy 2011). However, the lack of peck marks on the three artificial cowbird eggs that were accepted for the duration of one recording session suggest that gray catbirds did not attempt to puncture-eject them. Likewise, Lorenzana and Sealy (2001) did not find peck marks on model eggs that were accepted for 5 days. Yet, the recovered eggs in this study were in the nest for the duration of one recording session only and it is possible that the hosts may have tried to eject them later if they had been in the nest longer, which is likely because gray catbirds have been reported to take 2 to 3 days to eject in some cases (Lorenzana and Sealy 2001). Whether individual gray catbirds use one method of ejection exclusively or whether individuals possess the phenotypic plasticity to use one method or the other is not known.

The mechanism of detection in gray catbirds apparently is visual because in 8 of 15 ejections captured on video the ejection took place before the catbird sat on the nest. Warbling vireos also use visual cues because they ejected real cowbird eggs without first settling on the clutch, thus eliminating tactile cues (Underwood and

Sealy 2011). Likewise, Guigueno and Sealy (2012) suggested that the mechanism of detection in yellow warblers is visual because yellow warblers peered more at their eggs before settling on a clutch when a cowbird egg was present than when it was not. However, detection of parasitic eggs using olfactory cues cannot be ruled out because olfactory-mediated communication has been found to be important and widespread among birds (Roper 1999; Hagelin and Jones 2007; Balthazart and Taziaux 2009; Sealy and Underwood 2012).

Previous research has revealed that changes in incubation behaviour in response to parasitism is a good indication that hosts recognise that they have been parasitised because changes in incubation behaviour correlated with rejection behaviour in yellow warblers and rufous-tailed scrub wrens (i.e., time spent probing, Guigueno and Sealy 2012; time spent investigating the clutch, Soler et al. 2012). However, this relationship was not perfect and some individuals apparently recognised the eggs of parasites through changes in their incubation behaviour but ultimately accepted the egg (Antonov et al. 2009; Guigueno and Sealy 2012; Soler et al. 2012). The present study, to my knowledge, is the first study to test if a species that typically accepts parasitic eggs responds to artificial parasitism with changes in incubation behaviour. Red-winged blackbirds showed only a small change in probing frequency, which suggests they do not recognise when they have been parasitised, at least within the first three hours after parasitism.

Red-winged blackbirds may also respond to the eggs of cowbirds with changes in incubation behaviour beyond the three-hour observation period. It is possible that I did not detect a difference between trials where a cowbird was present and trials where a cowbird egg was not present because it takes more than three hours for female red-



winged blackbirds to recognise cowbird eggs or that their behaviour only changes in females that eventually reject parasitism.

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Table 3.1. Red-winged blackbird and gray catbird behaviour at the nest during “pre-cowbird egg” (PRE), “cowbird egg” (CBE), and “post-cowbird egg” (POST) observation periods. Values are reported as means  $\pm$  SE. P-values  $< 0.05$  were considered significant and are in bold.

Variable	Host species	n	PRE	CBE	POST	Friedman's ANOVA	
						$\chi^2$	p
Percentage of time present	Gray catbird	9	78.18 $\pm$ 2.44	85.37 $\pm$ 3.03	79.60 $\pm$ 2.05	6.00	<b>0.05</b>
	Red-winged blackbird	23	81.80 $\pm$ 1.73	84.22 $\pm$ 1.69	83.95 $\pm$ 1.38	0.26	0.88
Peers/h present at nest	Gray catbird	9	5.56 $\pm$ 1.15	10.21 $\pm$ 1.82	5.98 $\pm$ 0.94	4.67	0.10
	Red-winged blackbird	23	4.74 $\pm$ 0.40	4.76 $\pm$ 0.46	4.81 $\pm$ 0.37	1.13	0.57
Percentage of time peering when at nest	Gray catbird	9	0.26 $\pm$ 0.05	0.87 $\pm$ 0.18	0.35 $\pm$ 0.04	9.56	<b>0.01</b>
	Red-winged blackbird	23	0.26 $\pm$ 0.03	0.32 $\pm$ 0.07	0.27 $\pm$ 0.03	1.65	0.44
Probes/h present at nest	Gray catbird	9	9.68 $\pm$ 1.54	21.21 $\pm$ 3.90	9.76 $\pm$ 1.02	10.67	<b>0.01</b>
	Red-winged blackbird	23	7.44 $\pm$ 0.85	7.80 $\pm$ 0.59	6.55 $\pm$ 0.46	7.91	<b>0.02</b>
Percentage of time probing when at nest	Gray catbird	9	1.43 $\pm$ 0.24	5.50 $\pm$ 1.28	1.97 $\pm$ 0.34	14.00	<b>&lt;0.01</b>
	Red-winged blackbird	23	2.21 $\pm$ 0.47	2.17 $\pm$ 0.24	1.99 $\pm$ 0.30	5.30	0.07
On-bouts/h	Gray catbird	9	4.08 $\pm$ 0.54	4.14 $\pm$ 1.01	3.81 $\pm$ 0.56	0.41	0.81
	Red-winged blackbird	23	3.60 $\pm$ 0.24	3.58 $\pm$ 0.23	3.84 $\pm$ 0.27	0.29	0.86

Table 3.2. Post-hoc Wilcoxon matched-pairs tests showing the significance of differences between trials where significant differences in gray catbird incubation behaviour at the nest were found among the PRE, CBE and POST trials. P-values < 0.05 were considered significant and are in bold.

Variable	Wilcoxon matched pairs test (Z statistic), p		
	PRE vs. CBE	CBE vs. POST	PRE vs. POST
Percentage of time present at nest	2.67, <b>0.01</b>	2.07, <b>0.04</b>	0.53, 0.59
Percentage of time peering when at nest	2.55, <b>0.01</b>	2.19, <b>0.03</b>	1.48, 0.14
Probes/h present at nest	2.43, <b>0.02</b>	2.67, <b>0.01</b>	0.06, 0.95
Percentage of time probing when at nest	2.67, <b>0.01</b>	2.67, <b>0.01</b>	1.48, 0.14



Table 3.3. Red-winged blackbird behaviour at the nest according to type of egg manipulation during “pre-cowbird egg” (PRE), “cowbird egg” (CBE) and “post-cowbird egg” (POST) observation periods. Cowbird eggs were either added to blackbird nests, which increased the clutch size (Add) or switched with a host egg, which caused the clutch size to remain the same (Switch). Values are reported as means  $\pm$  SE.

Variable	Manipulation type	n	PRE	CBE	POST	Friedman's ANOVA	
						$\chi^2$	p
Percentage of time present at nest	Add	15	82.77 $\pm$ 8.99	85.34 $\pm$ 8.70	85.75 $\pm$ 6.73	0.93	0.63
	Switch	8	79.99 $\pm$ 7.00	82.13 $\pm$ 6.89	80.57 $\pm$ 5.18	3.25	0.20
Peers/h present at nest	Add	15	4.54 $\pm$ 1.81	4.53 $\pm$ 2.17	4.38 $\pm$ 1.59	0.93	0.63
	Switch	8	5.11 $\pm$ 2.12	5.20 $\pm$ 2.36	5.61 $\pm$ 1.91	1.00	0.61
Percentage of time peering when at nest	Add	15	0.25 $\pm$ 0.14	0.36 $\pm$ 0.38	0.28 $\pm$ 0.18	1.60	0.45
	Switch	8	0.28 $\pm$ 0.19	0.24 $\pm$ 0.09	0.27 $\pm$ 0.08	0.25	0.88
Probes/h present at nest	Add	15	8.14 $\pm$ 4.70	7.82 $\pm$ 2.93	6.80 $\pm$ 2.42	4.93	0.09
	Switch	8	6.13 $\pm$ 2.15	7.76 $\pm$ 2.49	6.07 $\pm$ 1.86	4.75	0.09
Percentage of time probing when at nest	Add	15	2.25 $\pm$ 1.91	2.22 $\pm$ 1.09	2.18 $\pm$ 1.63	2.80	0.26
	Switch	8	2.13 $\pm$ 2.95	2.06 $\pm$ 1.28	1.65 $\pm$ 0.93	3.25	0.20
On-bouts/h	Add	15	3.40 $\pm$ 0.95	3.32 $\pm$ 0.75	3.51 $\pm$ 1.09	0.67	0.72
	Switch	8	3.96 $\pm$ 1.46	4.08 $\pm$ 1.51	4.45 $\pm$ 1.46	0.75	0.69

## **Chapter 4: Do shining cuckoos *Chrysococcyx lucidus* produce preen wax that mimics the preen wax of their host?**

### ***4.1 Abstract***

Some avian brood parasites have evolved host egg and nestling mimicry to circumvent ever-improving discrimination by hosts. Visual and aural mimicry by avian brood parasites have been well documented, however, mimicry of host odours by avian brood parasites has not previously been investigated. Recent research has revealed that passerines may have well developed senses of olfaction and may use olfaction to gain spatial and temporal socio-biological information about their environment and about other individuals. One key source of avian body odour is uropygial gland secretions (preen wax). Preen wax composition has been found to vary among species, populations, and between the sexes. In this chapter, I compared the preen wax composition of the shining cuckoo *Chrysococcyx lucidus*, an avian brood parasite, the grey warbler *Gerygone igata*, the primary host of the shining cuckoo, and seven other potentially suitable host species. Preliminary assessment of gas-chromatography (GC-FID) traces suggest the preen wax composition of shining cuckoos is more similar to the preen wax composition of grey warblers than to any of the other seven species examined. This similarity was present in both adult and nestling shining cuckoos and grey warblers. Although alignment of peaks among

species using mass spectrometry data is required to confirm this finding, my results provide the first evidence for olfactory mimicry in any avian brood parasitic system.

## **4.2 Introduction**

Coevolution results from antagonistic and reciprocal interactions between two or more species where changes in one species select for changes in the second species which can select for further changes in the first species (Janzen 1980; Futuyma 1998). This pattern can continue in perpetuity or until some stable evolutionary end point is reached (Janzen 1980; Futuyma 1998). Some of the best examples of coevolution can be seen between brood parasites and their hosts (Rothstein and Robinson 1998). Failing to recognise and reject brood parasites is costly in term of reproductive output to the host, whereas failing to trick hosts to accept their young and adapt to their life histories is costly to the parasite (Lorenzana and Sealy 1999; Davies 2011). The reciprocal antagonistic interaction between parasites and their hosts, where parasites evolve better adaptations to fool hosts into raising their offspring and hosts evolve better discrimination (i.e., anti-parasite adaptations) to rid themselves of parasites, has been termed an “evolutionary arms race” (Dawkins and Krebs 1979; Rothstein and Robinson 1998).

Egg mimicry by the common cuckoo *Cuculus canorus* and egg discrimination by some of its hosts is one of the best examples of this “evolutionary arms race” (Davies 2000). Hosts use visual cues to detect parasitic eggs (Davies 2000; Avilés et al. 2010) and have been shown to discriminate against parasitic eggs based on their shape (Underwood and Sealy 2006; Guigueno and Sealy 2009), size (Marchetti 2000),

markings (Stoddard and Stevens 2010), and visible and ultra violet reflectance (Cherry et al. 2007b; Underwood and Sealy 2008; Avilés et al. 2010). In response, parasites may evolve visual egg mimicry to prevent hosts from detecting their eggs (Brooke and Davies 1988; Moksnes and Røskoft 1995; Honza et al. 2001) and female cuckoos may actually select nests with the best visual mimicry between their eggs and those of the host (Avilés et al. 2006; Cherry et al. 2007a). Previous studies have found that, intraspecifically, eggs that more closely mimicked the eggs of a host were rejected at a lower frequency than eggs that less closely mimicked the eggs of the same host (Davies and Brooke 1989; Moksnes et al. 1990; Welbergen et al. 2001; Bártol et al. 2002; Grim 2005; Cherry et al. 2007a).

Desertion or ejection of parasite nestlings by some hosts suggest the evolutionary arms race can continue beyond the egg stage (Langmore et al. 2003; Soler 2009; Sato et al. 2010; Tokue and Ueda 2010; Delhey et al. 2011; Grim 2011). For example, superb fairy-wrens *Malurus cyaneus* abandon 40% of nests containing a Horsfield's bronze-cuckoo *Chrysococcyx basalis* nestling and all nests containing a shining bronze-cuckoo *C. lucidus plagosus* nestling, despite never deserting nests containing one of their own nestlings (Langmore et al. 2003). Similarly, large-billed gerygones *Gerygone magnirostris* and mangrove gerygones *G. laevigaster* have been observed ejecting little-bronze cuckoo *C. minutillus* nestlings (Sato et al. 2010; Tokue and Ueda 2010).

Rejection of dissimilar nestlings by hosts has been suggested to select for the mimicry of nestling morphology and vocalisations in parasite species (Soler 2009) and comparative studies of nestling morphology as well as begging behaviour and calls provide compelling evidence for coevolution between hosts and parasites (Mundy 1973; Nicolai 1974; McLean and Waas 1987; Davies 2000; Avilés et al.

2010). In contrast to visual and auditory cues, the use of olfactory cues by host species to detect parasites has been poorly studied even though there is a growing body of evidence for highly acute functional olfactory systems in several biological contexts in many avian species (Roper 1999; Hagelin and Jones 2007; Balthazart and Taziaux 2009).

Historically, the role of olfaction in birds has been underestimated by biologists as it was believed to be restricted primarily to foraging in the Procellariiformes (Grubb 1972; Hutchison and Wenzel 1980; Nevitt 2000), kiwis (Wenzel 1968, 1971), New World vultures (Stager 1962) and honeyguides (Stager 1967; Smith and Paselk 1986). More recently, olfaction in birds has been discovered to be more important and widespread than previously thought (Roper 1999; Hagelin and Jones 2007; Rajchard 2007; Balthazart and Taziaux 2009) and present even passerines (Clark and Mason 1989; Clark et al. 1993; Steiger et al. 2008; Steiger et al. 2009). Despite having relatively small olfactory bulbs (Bang and Cobb 1968), passerines such as blue tits *Cyanistes caeruleus* have as many functional olfactory receptor genes as a snow petrel *Pagodroma nivea*, a Procellariiform (Steiger et al. 2008), which has one of the largest reported olfactory bulb to brain ratios among birds (Bang and Cobb 1968). Zebra finches *Taeniopygia guttata* also have extensive repertoires of intact olfactory receptor genes in their genome and these may be even larger than those of some reptile species (Steiger et al. 2009; Warren et al. 2010). By contrast, canaries *Serinus canaria* have far fewer functional olfactory receptor genes than blue tits, which suggests that olfactory abilities vary among passerines.

The use of olfaction in several behavioural contexts has recently been demonstrated in birds. For example, olfaction has been shown to be important in foraging Wandering albatross *Diomedea exulans* as they followed a flight pattern to

their prey that would be expected if a bird was using olfaction and not sight to find prey (Nevitt et al. 2008). Experiments testing the effect of odour (i.e., dimethyl sulphide) on foraging behaviour suggest many species of Procellariiformes use odour to find prey (Nevitt et al. 1995). However, the use of olfaction for foraging is not only restricted to the Procellariiformes. Common ravens *Corvus corax* have been shown to use olfaction when searching for carrion (Harriman and Berger 1986) and there is evidence that hummingbirds can distinguish between flowers by odour alone (Goldsmith and Goldsmith 1982; Ioalé and Papi 1989).

Olfaction may function in predator avoidance in some species. For example, house finches *Carpodacus mexicanus* reduced the time they spent on a feeder where predator faecal odour cues were present (Roth et al. 2008), crested auklets *Aethia cristatella* avoided mammalian musk odours in T-maze experiments in the laboratory (Hagelin et al. 2003), female dark-eyed juncos *Junco hyemalis* reduced incubation bout lengths in response to alien odours on their nest (Whittaker et al. 2009), and blue tits hesitated to enter their nests in the presence of chemical cues of a predator compared to a control (Amo et al. 2008). However, blue tits also hesitated to enter their nests after experimental manipulation with plant odours (Mennerat 2008) and eastern bluebirds *Sialia sialis* did not respond to the scent of a snake or of a mammal on their nest (Godard et al. 2007), suggesting the response to odours of predators may not always be universal.

Olfaction has also been shown to have a function in several other avian behaviours. Starlings *Sturnus vulgaris* and blue tits use olfaction to select nesting materials in order to create an aromatic environment (Clark and Mason 1985, 1987; Clark and Smeraski 1990; Malakoff 1999; Petit et al. 2002; Gwinner and Berger 2008; Mennerat 2008) that, in blue tits at least, has been found to be beneficial to

nestlings (Mennerat et al. 2009). Rock pigeons *Columba livia* use olfaction to detect odours that may be used for homing (Wallraff 2003, 2004; Gagliardo et al. 2009; Gagliardo et al. 2011). Likewise, some species of diving petrels (Pelecanoididae), storm petrels (Hydrobatidae), shearwaters and prions (Procellariidae) use odour to guide them to either their breeding islands, nesting colonies or their burrows (Grubb 1973, 1974, 1979; Benvenuti et al. 1993; Minguez 1997; Bonadonna et al. 2001; Bonadonna and Bretagnolle 2002; Bonadonna et al. 2003; Bonadonna and Nevitt 2004). Fledgling zebra finches also showed preference for the odour of their own nest over the odour of the nest of a conspecific, which suggests olfaction is important in nest identification in this colonially-nesting species (Caspers and Krause 2011).

Olfaction may also play a role in communication in some birds. Communication by olfaction is defined here as the detection and recognition of chemical odorants, and the transfer of information via chemical odorants (Kavaliers et al. 2005; Hagelin and Jones 2007). Individuals appear to gain important socio-ecological information on species, sex, and individual identity of other birds through olfaction (Mardon et al. 2010). For example, crested auklets preferentially orientated towards odours found on the feathers of conspecifics during the breeding season (Hagelin et al. 2003), although subsequent trials showed that males and females do not use odour when assessing mates (Jones et al. 2004). Antarctic prions *Pachiptila desolata* preferred the scent of conspecifics over their own scent, which is thought to have evolved to prevent inbreeding in this philopatric species, and they preferred their mate's scent over the scent of another conspecific, which is believed to have evolved to assist in finding their nest after being away foraging at sea for periods of up to 2 weeks (Bonadonna et al. 2007). Humboldt penguins *Spheniscus humboldti* were found to have the ability to detect and discriminate between familiar and non-familiar

individuals as well as kin and non-kin based on odour alone (Coffin et al. 2011). Female budgerigars *Melopsittacus undulatus* preferred the odour of males over the odour of females, and in a separate experiment, females showed a preference for males treated with octadecanol, nonadecanol, and eicosanol, three compounds found in uropygial secretions (Zhang et al. 2010). Olfaction may even play a role in parent-offspring recognition because ring doves *Streptopelia risoria* rejected their squabs when treated with a foreign odour (Cohen 1981); however, the results of this study are not conclusive since the odours the squabs were treated with (i.e., “fruit-scent”) are unlikely to be encountered by the parents at the nest in natural situations. Individual-specific odour and recognition may also be present in passerines because dark-eyed juncos may be able to detect the odour of conspecifics and heterospecifics at the nest (Whittaker et al. 2009). Zebra finches have also been shown to detect and discriminate between kin and non-kin based on odour (Krause et al. 2012).

The source of odours in birds may be substances produced by organs such as cloacal glands or epidermal cells which can produce volatile substances, at least in some species (Hagelin 2007b). However, a key source of avian body odours are uropygial gland secretions (hereafter, preen wax) because they contain volatile and non-volatile substances and they are spread over the feathers during preening (Hagelin and Jones 2007; Soini et al. 2007; Campagna et al. 2012). Preen wax is mainly composed of monoesters of fatty acids connected to long-chain alcohols by ester bonds (Thomas et al. 2010; Campagna et al. 2012). Also contained in the preen wax of some species are diesters, triesters, free alcohols, free fatty acids, tryglycerides, alkanes, alkenes, aldehydes, ketones, aliphatic acid, aromatic or cyclic molecules, amines and sulfites (Jacob 1976; Sweeney et al. 2004; Salibian and Montalti 2009; Thomas et al. 2010; Zhang et al. 2010; Campagna et al. 2012). The



diversity and complexity of the compounds found in preen wax makes the identification of individual compounds difficult (Thomas et al. 2010).

Preen wax is hypothesised to function in a variety of ways including maintaining feather flexibility (Jacob and Ziswiler 1982), preventing feather wear and providing waterproof protection (Elder 1954; Fabricius 1959; Stettenheim 1972; Elowson 1984; Sweeney et al. 2004), acting as a cosmetic (Piersma et al. 1999; Delhey et al. 2007; Piau et al. 2008), repelling or killing feather-degrading ectoparasites (Jacob and Ziswiler 1982; Jacob et al. 1997; Douglas et al. 2001; Moyer et al. 2003; Douglas 2004), and preventing microbial and fungal growth (Martín-Vivaldi et al. 2010). Uropygial secretions may also repel mosquitoes (Hwang et al. 1980; Kramer et al. 1980; Hwang et al. 1982; Douglas et al. 2005). It is also believed that in some species, the potent and unpleasant odour of the preen wax, such as the preen wax of the green woodhoopoe *Phoeniculus purpureus*, repels predators (Burger et al. 2004; Hagelin and Jones 2007).

Changes in preen wax composition affects the odour of preen wax and thus the odour of birds (Jacob et al. 1979; Whittaker et al. 2009; Whittaker et al. 2010; Shaw et al. 2011; Whittaker et al. 2011). Preen wax composition has been found to vary among species (Jacob and Ziswiler 1982; Sweeney et al. 2004; Haribal et al. 2005; Haribal et al. 2009; Whittaker et al. 2009; Zhang et al. 2009), populations (Whittaker et al. 2010) and individuals (De Leon et al. 2003; Bonadonna and Nevitt 2004; Bonadonna et al. 2007; Soini et al. 2007; Zhang et al. 2009; Caro and Balthazart 2010; Karlsson et al. 2010; Whittaker et al. 2010). Preen wax composition in some species varies with reproductive period and age (Kolattukudy and Sawaya 1974; Piersma et al. 1999; Sandilands et al. 2004; Reneerkens et al. 2007b; Soini et al. 2007; Whelan et al. 2010; Whittaker et al. 2010; Shaw et al. 2011; Amo et al. 2012), and

may also differ between the sexes (Jacob et al. 1979; Zhang et al. 2009; Mardon et al. 2010; Whittaker et al. 2010; Zhang et al. 2010; Whittaker et al. 2011; Amo et al. 2012). Furthermore, these variations have been found to be repeatable (Bonadonna et al. 2007; Whittaker et al. 2010) and important in intraspecific communication in seabirds (Hagelin et al. 2003; Bonadonna and Nevitt 2004; Bonadonna et al. 2007; Hagelin 2007a), penguins (Coffin et al. 2011), chickens (Hirao et al. 2009), and passerines (Whittaker et al. 2009; Whittaker et al. 2011; Amo et al. 2012).

As a result of the variability among and within species in the olfactory signals produced by preen wax (Soini et al. 2007; Shaw et al. 2011) and the demonstrated ability of some species to detect these odours (Whittaker et al. 2009), it is plausible that the olfactory signals produced by preen wax may act as cues for discrimination between host and brood parasite. In addition, sophisticated olfactory abilities have been demonstrated in the brown-headed cowbird *Molothrus ater*, an avian brood parasite (Clark and Mason 1989). However, the role of olfaction in the interaction between parasites and their hosts has never been investigated. Mimicry in egg appearance and nestling begging call structure is thought to have evolved in response to ever improving egg discrimination by hosts using visual and auditory cues, respectively (McLean and Waas 1987; Davies 2000; Anderson et al. 2009). Likewise, greater similarities in preen wax composition between parasites and their host species than between parasites and potentially suitable but non-used host species may indicate that some host species use chemical cues to detect and discriminate against parasitic young and that parasites evolved mimetic preen wax compositions to avoid detection at the nest.

In this chapter, I compared the preen wax composition of nestlings and adults of the shining cuckoo *Chrysococcyx lucidus*, a brood parasite, grey warbler

*Gerygone igata*, the primary host of the shining cuckoo, and six potentially suitable but non-used host species in New Zealand for evidence of mimicry. The escalation of the evolutionary arms race, to the point where discrimination of nestlings is based on odour, seems plausible in the host-specialist shining cuckoo and its host, the grey warbler, because shining cuckoos seem to have won the evolutionary arms race at every stage. Grey warblers do not reject parasitic eggs perhaps indicating that shining cuckoos have won the evolutionary arms race at the egg stage (Briskie 2003; Grim 2011). The dark interior of the domed nests of grey warblers may make it difficult for grey warblers to see the cryptic green eggs of the shining cuckoo (Briskie 2003; Grim 2011). Also, the remarkable similarity in appearance of shining cuckoo and grey warbler nestlings as well as the similarities in their begging calls suggests grey warblers may have discriminated against nestlings unlike their own yet shining cuckoos are apparently winning the arms race at this stage as well because grey warblers have not been observed rejecting shining cuckoo nestlings (McLean and Waas 1987; Anderson et al. 2009). However, two congeneric species with the grey warbler have been shown to reject the nestlings of the *plagosus* subspecies of the shining cuckoo (i.e., shining-bronze cuckoo) and the Horsfield's bronze cuckoo in Australia (Sato et al. 2010; Tokue and Ueda 2010). Being unable to discriminate between parasites and their own young based on sight and sound, grey warblers may have instead evolved discrimination based on odour. In addition to comparing the preen wax composition of nestlings, I compared the preen wax composition of adults to determine if it changes with age and if there is evidence for host-mimicry in adult shining cuckoos. Mimicry in an adult host-specialist brood parasite may be important for avoiding detection at the nest.

Ideally, experiments testing the effect of odour on nestling discrimination are required to support mimicry (Grim 2011). Nevertheless, identifying a close correspondence between the composition of preen waxes of a brood parasite and its host, but not between the parasite and other non-host species, would be consistent with the evolution of odour mimicry. I present here a comparative study of the preen wax composition of parasites, hosts and non-used but potentially suitable host species as a first step towards understanding the role of olfaction in avian brood parasitism. This study is the first to investigate the possible role of olfaction in avian brood parasite systems.

### **4.3 Methods**

#### *Study sites and species*

Preen wax samples were collected from nestlings and adults of the shining cuckoo, grey warbler and non-used but potentially suitable host species present in Kowhai Bush (42°23' S, 173°37' E) and Waimangarara Bush (42°20' S, 173°40' E), which are both located approximately 10 km from Kaikoura, New Zealand. Included in the analyses are 147 preen wax samples that were collected between 7 October and 11 December 2011 and between 19 September and 17 December 2012. Samples were collected from nestlings and adults of five and nine species, respectively (Table 4.1). Although I only compared the preen wax composition of shining cuckoos to six native passerine species, these species, with the exception of rifleman *Acanthisitta chloris*, are the most abundant native passerine species in New Zealand, and they represent 38.8% of terrestrial native passerine species in New Zealand. The native passerine

species that were not sampled because they were either not present on the study site, or rare on the study site are the rock wren *Xenicus gilviventris*, fernbird *Bowdleria punctata*, yellowhead *Mohoua ochrocephala*, whitehead *Mohoua albigilla*, tomtit *Petroica macrocephala*, kokako *Callaeas cinerea*, tui *Prothemadera novaeseelandiae*, stitchbird *Notiomystis cincta* and saddleback *Philesturnus carunculatus*. I also compared the preen wax composition of the shining cuckoo to the preen wax composition of the introduced song thrush *Turdus philomelos*.

Adults were captured with mist nets and traps. Nestlings were handled at the nest and preen wax samples were collected between the day their primary feathers emerged from their sheaths and four days later (Briskie et al. 1999). Preen wax samples were collected from one randomly selected nestling per nest to avoid pseudoreplication and to minimise disturbance at nests. All sampled adults and nestlings were fitted with metal bands from the Department of Conservation (New Zealand). All sampled adults were sexed (based on morphology and/or colouration) and their breeding status was assessed (based on known nests and/or the presence of brood patches and enlarged cloacal protuberances). Only adults in breeding condition were included in the study. Shining cuckoos were not sexed and their breeding status was not assessed. However, I assumed all individuals sampled were breeding since they were captured during the peak of their breeding period (i.e., from late October to late December). I did not determine the sex of any of the nestlings sampled for this study.

#### *Sample collection and storage*

The area around the preen gland was gently massaged with wax-tipped forceps until a small amount of preen wax was discharged. I collected the preen wax on a sterile

inoculation loop (Kolattukudy et al. 1987; Soini et al. 2007; Fluen 2008). Inoculation loops were placed in sterile  $\mu$ Vial glass inserts from Gerstel Inc. that were placed in sterile, airtight dark-brown glass vials. Springs were placed in the bottom of the dark-brown glass vials to push the openings of the  $\mu$ Vial against the inside of the lid when closed. Vials with dark-brown glass were used to reduce the possible effect of light on preen wax composition. The samples were stored at  $-20^{\circ}\text{C}$  until extraction and analysis by gas chromatography-flame ionization detector (GC-FID)(Soini et al. 2007; Fluen 2008). To prevent contamination of the samples, birds were handled with latex gloves and the portion of the inoculation loop that was handled with latex gloves was cut off when inserting the sample in the glass vials. The wax-tipped forceps and the cutting edges of the pliers used to cut the inoculation loop were cleaned with ethanol (90%) between every sample. A series of loops in which we did not collect preen wax were run through the entire preen wax collection protocol and analysed with the GC-FID. These trials confirmed the profiles represent preen wax and not any non-bird odours.

#### *Inoculation loop and glass vial-cleaning procedures*

Glass vials were thoroughly cleaned to remove all substances that could be detected by thermo-desorption method on a GC-MS. Test tubes and glass vials were first soaked for several hours in hot water containing a strong lab detergent. They were then rinsed clean deionised water to remove all detergent. A fine water jet was used to rinse the inside of the  $\mu$ Vials and the dark-brown glass vials. They were then rinsed sequentially with three organic solvents: methanol, acetone, and n-hexane.  $\mu$ Vials and the dark-brown glass vials were then baked overnight in an oven at  $230^{\circ}\text{C}$  before being assembled with forceps that were cleaned using the same procedure. The lids

were rinsed with deionised water and ethanol, and then air-dried. Lids were not washed with detergent because of the risk of detergent remaining behind the lid gasket.

#### *Preparation of preen wax samples for GC-FID*

Samples were removed from the freezer on the day they were analysed. Latex gloves were worn when handling samples to prevent contamination. New caps for the glass vials were prepared by inserting a new PTFE septum into a new cap. The old cap was removed from the glass vial containing the sample. The preen wax on the inoculation loop was diluted in ethyl acetate by adding 100  $\mu$ l of ethyl acetate to the  $\mu$ Vials containing the inoculation loop. The ethyl acetate that was used to dilute the samples was stored at -20°C until required. A new and sterile pipette tip (P200) was used for each vial when adding the ethyl acetate to prevent contamination. Once the ethyl acetate was added, the new caps were put on the vials. The vials were then agitated for 60 s to dissolve the preen wax off the loop. The vials were then opened and the wire loop was removed and discarded using sterile forceps. The cap was then replaced and the sample was loaded into the GC.

#### *Chemical analysis*

The method used in this study is similar to the method used previously by others such as Dekker et al. (2000) and Reneerkens et al. (2005) to analyse bird preen wax. An AOC-20 auto injector was used to inject 1  $\mu$ l of diluted preen wax from each sample into a Shimadzu GC-2010 gas chromatograph. The syringe of the auto injector was rinsed three times pre- and post-run with the solvent (ethyl acetate) and was rinsed once with the sample prior to injection. Split injection mode (6:1) was used carrying

nitrogen (carrier gas) at a flow of 19.0 mL/min and at a set pressure of 57.7 kPa. The temperature profile used had an initial temperature of 70°C, which rose at a rate of 20°C/min to 130°C. The temperature then rose at a rate of 4°C/min to a final temperature of 320°C, which was held for 15 min. The duration of the total temperature programme described above was 70 min. The flame ionisation detector (FID) was set to 320°C and at a sampling rate of 40 msec.

#### *Post-processing of gas chromatography data*

Within-species retention time shifts were aligned with the *icoshift* algorithm (version 1.2) (Savorani et al. 2010; Tomasi et al. 2011) in MATLAB (The Mathworks Inc., Natick, Massachusetts, USA). *icoshift* was designed specifically to deal with signal alignment problems in NMR data (Savorani et al. 2010). However, it can also deal with other spectra-like data sets such as chromatographic data (Tomasi et al. 2011). Among-species alignment of gas chromatography data has not yet been conducted. Baseline subtractions were conducted in R (R Team 2008) using the ‘*rmbaseline*’ function in the ‘*PROcess*’ package (version 1.36.0; Li 2005). Peak selection and integration was conducted in R using the ‘*isPeak*’ function in the ‘*PROcess*’ package (version 1.36.0; Li 2005). Data were then normalised giving a sum of unity to all abundance readings for each sample.

#### *Statistical analyses*

##### *Host-odour mimicry in the shining cuckoo*

Statistical analyses that assess the significance of the differences between the chemical profiles of different species are not possible unless gas chromatography data can be aligned among species. Among-species alignment requires the identification of



the compounds represented by several of the peaks for each species. This requires running a subsample of the preen wax samples through a gas chromatographer-mass spectrometer (GC-MS). The identified peaks can then be used as points of reference to align the gas chromatography data among species. GC-MS has not been completed for this study and as a result, comparisons using only results from GC traces that I report here should be viewed as preliminary until alignment between species can be confirmed with mass spectrometry data. Nonetheless, a comparison of non-aligned profiles provides an informative first stage towards assessing the presence (or otherwise) of mimicry in this host-parasite system.

As profiles between species could not be aligned, some profiles are likely shifted to the right or the left of each other. This shift could reflect real differences in the composition of preen waxes between two species, or it could instead reflect the lack of calibration, in which the peaks of two species are indeed identical compounds but one is shifted in time relative to the other. The latter seems likely when a large number of peaks in one species appears to correspond to a similar number of peaks in a second species but in which most (or every) one of the peaks are shifted the same distance in one direction. It seems more likely that the shift is due to calibration error and not that species 2 by coincidence has compound that differ in the exact fashion as species 1. Eventually, this assumption can be tested and confirmed with mass spectrometry data.

Although the total duration of each GC run spanned 2560 s, here I only report the first 1280 s of each profile in the chromatograms to show the details of the preen wax profiles more clearly and to facilitate comparison with the preen wax profile of the shining cuckoo. The retention times of 30 largest peaks of the preen wax profiles of adult and nestling shining cuckoos were all less than 1280 s, thus this truncation

does not ignore any compounds detected after 1280 s. Despite not being aligned, visual assessment of these profiles hints at the possibility of odour mimicry between grey warblers and shining cuckoos. This suggestion is tentative until the proper alignment and statistical tests can be conducted. Whether this shift in retention times is real or is due to instrument error, variations in the conditions during sample analysis, and/or post-processing manipulations is not known. However, the identification of the compounds represented by some of the peaks will eliminate this unknown and peaks will be aligned with confidence. Before proceeding to publication the mass spectrometry analyses will be complete, the preen wax profiles will be aligned, and statistical tests will be conducted.

Visual assessment of similarity between the preen wax profiles was based on the presence or absence of peaks in the mean preen wax profile of the species being compared that have similar heights and similar retention times to 12 of the largest peaks I selected on the mean preen wax profile of adult shining cuckoo (Fig. 4.1) or nestling shining cuckoos (Fig. 4.2; peaks numbered on the chromatograms). I chose these peaks because they were large and easily distinguishable from other peaks. To facilitate visual assessment of the similarity of preen wax profiles, chromatograms indicating the mean relative abundance of compounds in preen wax according to their retention times were constructed for the breeding males, breeding females, and nestlings of each species for which samples were taken. On each of these chromatograms, I superimposed the mean preen wax profile of the shining cuckoo. I compared the mean preen wax profiles of adults and nestlings separately. The mean preen wax profiles of adult males and females of each species were compared separately to the mean preen wax profile of adult shining cuckoos of unknown sex (Figs 4.3–4.17). The mean preen wax profile of nestlings was compared to the mean

preen wax profile of nestling shining cuckoos (Figs. 4.18–4.21). ‘Corresponding peaks’ are defined here as peaks that have heights that are within 50% of the height of a shining cuckoo peak and that are offset by no more than 60 s from the shining cuckoo peak. In addition, all ‘corresponding peaks’ for a species must be shifted to one side of all of the shining cuckoo peaks (i.e., all corresponding peaks for a species must be found to the left or to the right of the shining cuckoo peaks) and they must all be shifted by approximately the same amount of time. Although this method of comparison may seem somewhat arbitrary, it is based on the assumption that if, for example, two species both have 12 peaks and that the 12 peaks of one species are shifted the same time interval to the left of the other species, then it is likely the peaks in both species represent the same compounds and that the shift is due to lack of calibration.

*Within-species differences in preen wax composition between sexes and age groups*

The areas under each peak were used for within-species preen wax profile comparisons between breeding females, breeding males and nestlings. I was unable to sample preen wax from the nestlings of all species and thus only comparisons between breeding females and breeding males were conducted for some species. The areas under the peaks were normalised by dividing the area under a peak by the sum of the area for all of the peaks for each sample. Peaks were then analysed by principal component analyses to reduce the total number of variables that would be included in a subsequent discriminant analysis. A discriminant analysis was then conducted for each species to determine if breeding females, breeding males and nestlings could be discriminated based on their chemical profiles (Foitzik et al. 2007). The same statistical procedures will be used to determine if species can be discriminated based

on their chemical profiles and to assess the degree of similarity between shining cuckoos and grey warblers. Shapiro-Wilk's W tests were used to assess the normality of each dataset (Shapiro et al. 1968). The STATISTICA 12.0 (Statsoft, Tulsa, OK) software application was used to conduct all statistical analyses. All tests with P-values  $\leq 0.05$  were considered significant and values are represented as means  $\pm$  SE.

#### **4.4 Results**

##### *Host-scent mimicry in the shining cuckoo*

Visual assessment of the similarity in the shape of the preen wax profiles suggests that the preen wax profile of grey warblers and shining cuckoos are more similar to each other than to any other species I sampled (Figs 4.1–4.21; Table 4.1). The preen wax profile of the brown creeper *Mohoua novaeseelandiae* also showed a striking similarity to the preen wax profile of the shining cuckoo, but it was not as similar as the grey warbler preen wax profile was to the shining cuckoo preen wax profile (Figs 4.1–4.21; Table 4.1).

##### *Breeding adults*

The overall shape of the preen wax profiles of breeding male and breeding female grey warblers are very similar to the overall shape of the preen wax profile of adult shining cuckoos. The preen wax profiles of breeding male and breeding female grey warblers have peaks that correspond to all of the 12 marked peaks in the adult shining cuckoo preen wax profile except for peak 12 (Figs. 4.3, 4.4; Table 4.1). However, the

preen wax profile of grey warblers appears to be shifted approximately 30 s to the left relative to the shining cuckoo profile.

The overall shape of the preen wax profiles of breeding male and female brown creepers and breeding male and female bellbirds are also similar to the shape of the preen wax profile of adult shining cuckoos (Figs. 4.5, 4.6, 4.13, 4.14). However, the preen wax profiles of breeding male and female brown creepers have peaks that correspond with only 7 of the 12 marked peaks in the adult shining cuckoo preen wax profile (Figs. 4.5, 4.6; Table 4.1) and breeding male and female bellbirds have peaks that correspond with only 6 and 5 of the 12 marked peaks in the adult shining cuckoo preen wax profile, respectively (Figs 4.13, 4.14; Table 4.1). The preen wax profiles of breeding male rifleman have peaks that corresponded with 5 of the 12 marked peaks in the adult shining cuckoo preen wax profile (Fig. 4.11; Table 4.1). The preen wax profiles of breeding adults for all of the other species that were sampled have three or less peaks that correspond with the 12 marked peaks on the adult shining cuckoo preen wax profile (Figs. 4.7, 4.8, 4.9, 4.10, 4.12, 4.15, 4.16, 4.17; Table 4.1).

### *Nestlings*

The preen wax profile of grey warbler nestlings appears more like the preen wax profile of nestling shining cuckoos than the preen wax profiles of nestling South Island fantails *Rhipidura fuliginosa fuliginosa* (hereafter fantail), New Zealand robins *Petroica australis* or silvereyes *Zosterops lateralis* (Table 4.1). The preen wax profile of nestling grey warblers has peaks that corresponded with 10 of the 12 marked nestling shining cuckoo peaks whereas the preen wax profiles of fantail, New Zealand robin and silvereye nestlings have only zero, one and three peaks that corresponded with the 12 marked nestling shining cuckoo peaks, respectively (Table 4.1).

*Within-species differences in preen wax composition between sexes and age groups**Shining cuckoo*

Discriminant analyses correctly classified the preen wax samples of shining cuckoos according to nestling or adult (Table 4.2).

*Grey warbler*

When all peaks were included in the analysis, discriminant analysis correctly classified 14 of 16 grey warbler males, 6 of 9 grey warbler females, and all grey warbler nestlings based on the gas chromatography profiles of their preen wax (Table 4.2). One grey warbler male was misclassified as a female and another grey warbler male was misclassified as a nestling. Three grey warbler females were misclassified as males. When only the 30 largest peaks were included in the analysis, discriminant analysis correctly classified 13 of 16 grey warbler males, 6 of 9 grey warbler females, and 4 of 5 nestlings based on the gas chromatography profiles of their preen wax (Table 4.2). Two grey warbler males were misclassified as females and one grey warbler male was misclassified as a nestling. Three grey warbler females were misclassified as males. One grey warbler nestling was misclassified as a male.

*Bellbird*

When all peaks and when only the 30 largest peaks were included in the analysis, discriminant analyses correctly classified 12 of 13 bellbird males and 13 of 16 bellbird females based on the gas chromatography profiles of their preen wax (Table 4.2).

*Fantail*

Discriminant analyses failed classify fantail preen wax profiles according to sex and age group (Table 4.2). Discriminant analyses correctly classified zero of three males, 10 of 12 females, and three of five nestlings when all peaks were included in the analyses. However, discriminant analyses misclassified the preen wax profiles of three males as females, two females as nestlings, and two nestlings as females. When only the 30 largest peaks were used, discriminant analyses correctly classified zero of three males, 10 of 12 females, and one of five nestlings but misclassified the preen wax profiles of three males as females, two females as a nestlings, and four nestlings as females. The results were not significant (Table 4.2).

*New Zealand robin*

When all peaks were used, discriminant analyses correctly classified seven of nine males, four of seven females, and five of six nestlings but misclassified the preen wax profiles of two males as females, one female as a male, two females as nestlings, and one nestling as a female (Table 4.2). When only the 30 largest peaks were used, discriminant analyses correctly classified seven of nine males, two of seven females, and four of six nestlings and misclassified two males as females, two females were as males, two other females as nestlings, and two nestlings as females (Table 4.2).

*Rifleman*

Discriminant analyses correctly classified three of four females and three of four males when all peaks were used (Table 4.2). Discriminant analyses correctly classified four of four males but only two of four females when only the 30 largest peaks were used (Table 4.2). However, the non-significance of the results of discriminant analyses and misclassification rates suggest breeding males, breeding

females and nestlings of fantails and rifleman cannot be discriminated on the basis of their preen wax profiles (Table 4.2).

#### *Brown creeper*

When all peaks were used in the analysis, discriminant analyses correctly classified three of three males and six of six females based on gas chromatography traces of their preen wax (Table 4.2). The results, however, were not significant (Table 4.2).

When only the 30 largest peaks were used in the analysis, discriminant analyses again correctly classified preen wax profiles according to sex and the results were significant (Table 4.2).

#### *Silvereye*

When all the peaks were used in the analysis, discriminant analyses correctly classified all nine males and one of two females (Table 4.2). The results, however, were not significant (Table 4.2). However, when only the 30 largest peaks were used in the analysis, discriminant analyses correctly classified all preen wax profiles according to sex and the results were significant (Table 4.2).

### **4.5 Discussion**

The results indicate that mimicry of the composition of grey warbler preen wax by shining cuckoos is likely. However, this conclusion should be viewed as tentative because it is based on preen wax profiles that have not been aligned with mass spectrometry. Alignment of peaks among species followed by statistical tests are required to properly assess the degree of similarity of the preen wax profiles of the shining cuckoo and each of the species included in this paper. Alignment among



species requires knowing the identity of the compounds represented by the peaks in the gas chromatography traces, which can be achieved by analysing a subsample of preen wax samples for each species using a mass spectrometer. Nevertheless, alignment is likely to shift the peaks in the profiles by  $< 60$  s and thus the similarity of profiles between species are likely to be real and not indicative of different compounds.

The results also suggest that preen wax composition varies with sex and age in five of the eight native New Zealand species tested here (Table 4.2). This finding, however, has previously been seen in other species. For example, intersexual differences in preen wax composition were also found in domesticated Bengalese finches *Lonchura striata* (Zhang et al. 2009; Zhang et al. 2010), hoopoes *Upupa epops* (Martín-Vivaldi et al. 2009), and dark-eyed juncos (Soini et al. 2007; Whittaker et al. 2010; Whittaker et al. 2011). However, preen wax composition does not vary between the sexes in some other species. For example, preen wax composition did not differ significantly between the sexes in rock doves *Columba livia*, but the samples that were compared were taken during the non-breeding season (Montalti et al. 2005). Similarly, semivolatile compounds did not differ significantly between the sexes in adult gray catbirds *Dumetella carolinensis* (Shaw et al. 2011).

The results also suggest the preen wax composition of nestlings differs significantly from the preen wax composition of adults in shining cuckoos and grey warblers but that the nestlings of fantails and New Zealand robins could not be discriminated by preen wax composition with a high level of certainty. The finding that the preen wax composition of nestlings differs from that of the adults is not new. For example, the preen wax composition of chickens changes significantly in terms of

chain length and diastereomer composition with age from 3 to 13 months, after which it changes very little (Kolattukudy and Sawaya 1974).

If statistical tests confirm the composition of grey warbler and shining cuckoo preen wax are more similar to each other than to the preen wax composition of other sympatric species, which seems likely based on the similarity of the gas chromatography traces, it would be the first indication of an evolutionary arms race occurring between avian brood parasites and their hosts on the sense of olfaction. If grey warblers and shining cuckoos are found to have preen wax profiles that are more similar to each other than to any other species, experiments testing the abilities of grey warblers to discriminate against nestlings treated with preen wax from different species will be required to determine if variations in the relative abundance of certain compounds contained in preen wax are used to discriminate against foreign nestlings and if nestling discrimination by hosts is a selective pressure for the evolution of preen wax composition mimicry in the shining cuckoo (Grim 2011). Discrimination of nestlings with altered odours by grey warblers is likely, because congenetics have been observed ejecting parasite nestlings from the nest (Sato et al. 2010; Tokue and Ueda 2010) and because I observed a female grey warblers eject a live day-old fantail nestling from the nest (pers. obs.). However, it is not known if odour, visual appearance or vocalisations was used by the grey warbler to discriminate the fantail nestling and this requires further testing.

The similarity between the shining cuckoo and the brown creeper preen wax traces is puzzling because there are no records of shining cuckoos parasitising brown creepers (Gill 1998). The similarity could be due to phylogeny, but this is unlikely as shining cuckoos, grey warblers and brown creepers are not particularly close relatives. Future research needs to address this similarity.

The high individual repeatability of preen wax composition suggests it may be correlated with genotypes (Whittaker et al. 2010), but as both grey warblers and shining cuckoos occur in the same habitat, environmental effects cannot be ruled out as an explanation for some of the similarity in preen wax odours. In addition, the effect of selective pressures other than discrimination by hosts need to be investigated such as ectoparasite load (Galván et al. 2008) and predation pressures at the nest (Reneerkens et al. 2002, 2005; Reneerkens et al. 2007a; Reneerkens et al. 2007b).

Diet has been found to affect preen wax composition (Sandilands et al. 2004; Haribal et al. 2005; Thomas et al. 2010; Shaw et al. 2011) and may account for differences in preen wax composition among the species examined and may also be responsible for the similarity between the preen wax compositions of shining cuckoo and grey warbler nestlings since they may be fed similar diets from their grey warbler parents. For example, benzyldehyde, which is found in stone fruits such as black cherries, which are eaten by gray catbirds, was found in the preen wax of wild gray catbirds but not in the preen wax of captive gray catbirds which were not fed stone fruit (Shaw et al. 2011). Diet was also found to affect preen wax composition in white-throated sparrows *Zonotrichia albicollis* (Thomas et al. 2010). The preen wax of captive white-throated sparrows fed a diet enriched with sesame oil had a higher weighted mean monoester carbon number than those fed a diet enriched with fish oil. However, since fatty acids found in preen wax are known to be synthesized in the uropygial gland from acetate and propionate precursors (Jacob and Ziswiler 1982; Stevens 1996) the mechanism involved is likely indirect and complex (Thomas et al. 2010). Likewise, the fatty acid profile of the preen wax of rock doves was found to be different from the fatty acid profile of the diet fed to the same rock doves (Montalti et al. 2005). Diet also had no effect on preen wax composition in dark-eyed juncos

because the preen wax composition of captive and wild dark-eyed juncos were very similar despite differences in diet (unpubl. data in Whittaker et al. 2009). More experiments testing the overall effect of diet on avian body odour as well as the possible socio-ecological effects caused by diet-modified preen wax are required.

A better understanding of preen wax composition and how it translates to odour is required, especially since some of the compounds found in preen wax may be transformed after being applied to the feathers (Campagna et al. 2012). Differential preen wax transformation on the feathers among species may give birds different odours despite having similar preen wax composition. Similarity between gas chromatography traces of the compounds found on feathers and in preen wax of the same individual suggests that the likely source of these compounds is the uropygial gland, which is not surprising because birds spread preen wax on their feathers while preening (Sandilands et al. 2004; Soini et al. 2007; Zhang et al. 2009; Mardon et al. 2011). However, several significant differences exist between the compounds found on the feathers and in preen wax (Soini et al. 2007; Campagna et al. 2012). It has been hypothesized that these differences are the result of bacterial degradation and chemical conversions (Soini et al. 2007; Campagna et al. 2012). It has also been hypothesised that some of the compounds found on the feathers may have come from different sources such as the epidermis or the environment (Campagna et al. 2012). One possible source of odour that may affect the odour of nestlings is the transfer of scent from a parent or foster parent. Whittaker et al. (2009) hypothesised that the acceptance of brown-headed cowbirds by hosts may be because the host's scent is transferred to the young parasite. A better understanding of the final contribution of preen wax, as well as the contribution of other potential odorants from different

sources, to overall avian body odour is required to better understand the possible role of olfaction in avian host-parasite relationships.

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Table 4.1. Interspecies comparison of gas chromatography (GC-FID) traces of the preen wax of nine species sampled at Kowhai and Waimangara Bushes, South Island, New Zealand. GC-FID traces have not yet been aligned among species but will be aligned once mass spectrometry analyses are complete. Provisionally, I present a subjective assessment of the similarity of the traces between species. The number of corresponding peaks that match refers to the number of peaks that matched the 12 main peaks in the GC profiles of the shining cuckoo.

Species	n	Retention times (s)			"Corresponding" peaks	
		mean	min	max	Matches	Matching peak ID
Shining cuckoo						
Adult	10	768.304	527.20	980.88	n/a	n/a
Nestling	3	647.040	356.72	910.96	n/a	n/a
Grey warbler						
Breeding female	9	676.432	422.24	885.6	11	1,2,3,4,5,6,7,8,9,10,11
Breeding male	16	666.532	422.24	885.6	11	1,2,3,4,5,6,7,8,9,10,11
Nestling	5	630.420	334.88	885.6	10	1,2,4,5,6,7,8,9,11,12
Bellbird						
Breeding female	16	870.432	734.52	978.24	6	7,8,9,10,11,12
Breeding male	13	852.804	712.88	1027.28	5	7,8,9,10,12
Fantail						
Breeding female	12	819.860	699.16	954.32	3	7,10,11
Breeding male	3	819.860	699.16	954.32	3	7,10,11
Nestling	5	846.216	699.16	994.44	0	

[table continues on next page]

Table 4.1. [continued]

New Zealand robin						
Breeding female	7	563.700	190.64	881	3	3,6,10
Breeding male	9	779.360	195.32	1244.32	0	
Nestling	6	534.184	377.68	868.76	1	6
Rifleman						
Breeding female	4	681.784	512.36	825.28	4	2,4,7,10
Breeding male	4	796.652	595.60	986.48	5	4,7,8,10,11
Brown creeper						
Breeding female	4	821.500	624.72	992.32	7	4,5,7,8,9,10,11
Breeding male	6	803.852	624.72	992.32	7	4,5,7,8,9,10,11
Silvereye						
Breeding female	2	946.012	805.28	1089.32	4	7,8,9,12
Breeding male	9	985.992	728.24	1159	3	8,9,10
Nestling	1	1006.112	805.28	1159	3	7,8,9
Song thrush						
Adult	3	1253.564	1104.6	1372.12	0	

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Table 4.2. Results of discriminant analyses for intraspecific differences among sex and age groups for eight native species in Kowhai and Waimangara Bushes, South Island, New Zealand.

Species	n	# peaks used in analyses	Wilk's $\lambda$	F*	p	N correctly assigned/N total			
						Adults	Male	Female	Nestling
Shining cuckoo	13	72	0.072	18.08 (5,7)	0.0007	10/10	-	-	3/3
		30	0.138	12.52 (4,8)	0.0016	10/10	-	-	3/3
Grey warbler	30	75	0.168	4.31 (14,42)	0.0001	-	14/16	6/9	5/5
		30	0.199	7.44 (8,48)	0.0000	-	13/16	6/9	4/5
Bellbird	29	63	0.410	6.61 (5,23)	0.0006	-	12/13	13/16	-
		30	0.396	7.01 (5,23)	0.0004	-	12/13	13/16	-
Fantail	20	59	0.684	1.04 (6,30)	0.4170	-	0/3	10/12	3/5
		30	0.776	1.08 (4,32)	0.3818	-	0/3	10/12	1/5
New Zealand robin	22	59	0.485	3.92 (4,36)	0.0096	-	7/9	4/7	5/6
		30	0.514	3.56 (4,36)	0.0152	-	7/9	2/7	4/6

[table continues on next page]

Table 4.2 [continued]

Rifleman	8	92	0.447	1.65 (3,4)	0.3127	-	3/4	3/4	-
		30	0.552	1.08 (3,4)	0.4518	-	4/4	2/4	-
Brown creeper	10	71	0.295	3.99 (3,5)	0.0852	-	6/6	3/3	-
		30	0.090	16.77 (3,5)	0.0048	-	6/6	3/3	-
Silvereye	11*	107	0.316	3.25 (4,6)	0.0962	-	9/9	1/2	-
		30	0.160	7.86 (4,6)	0.0145	-	9/9	2/2	-

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\*Preen wax profile of silvereye nestling not included because n = 1.

Figure 4.1. Mean gas chromatogram of the preen wax collected from ten adult shining cuckoos captured in Kowhai Bush, South Island, New Zealand.

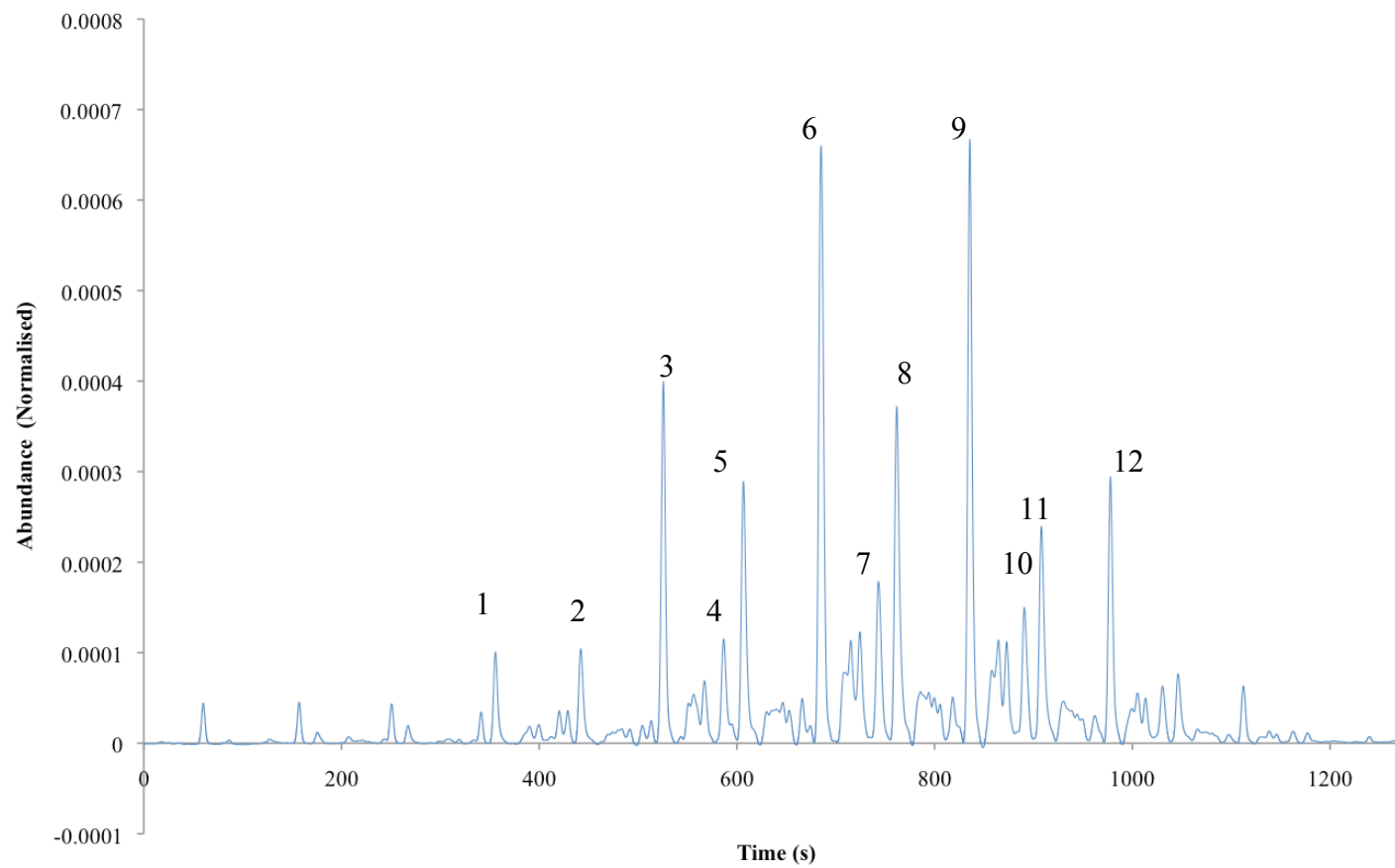


Figure 4.2. Mean gas chromatogram of the preen wax of three nestling shining cuckoos sampled near Kaikoura, South Island, New Zealand.

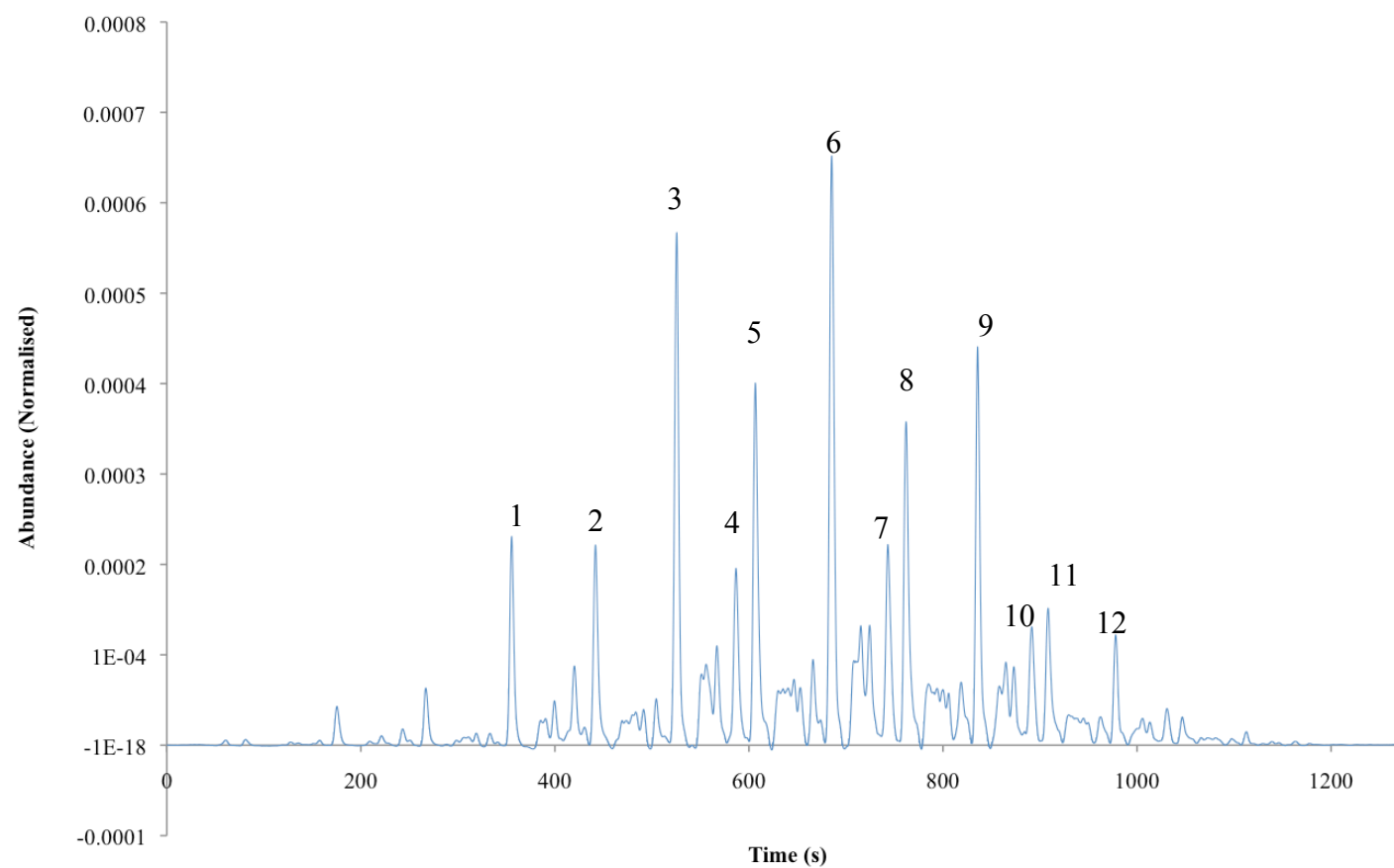


Figure 4.3. Mean gas chromatogram of the preen wax of breeding male grey warblers (red;  $n = 13$ ) and of adult shining cuckoos (blue;  $n = 10$ ).

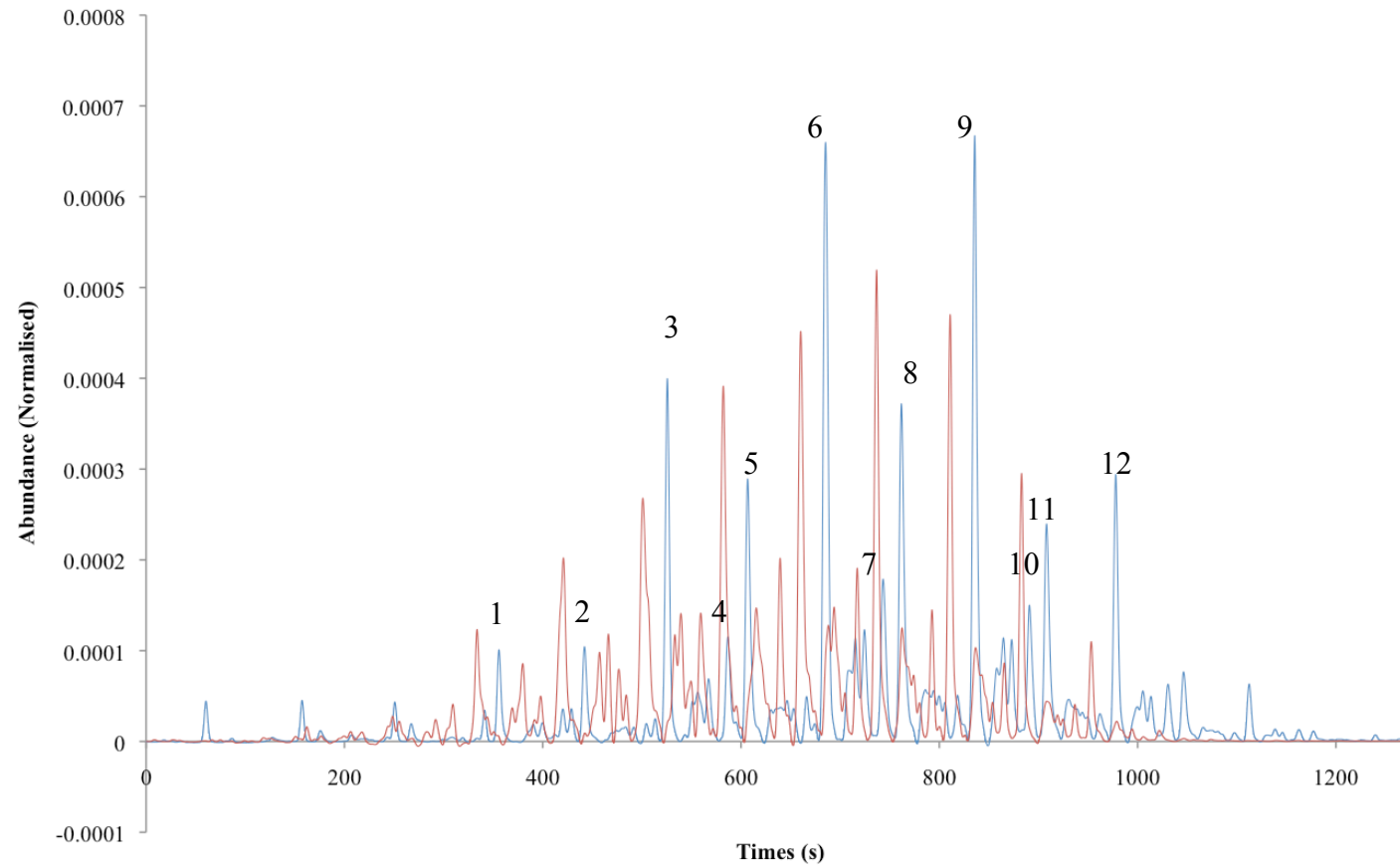


Figure 4.4. Mean gas chromatogram of the preen wax of breeding female grey warblers (red;  $n = 16$ ) and of adult shining cuckoos (blue;  $n = 10$ ).

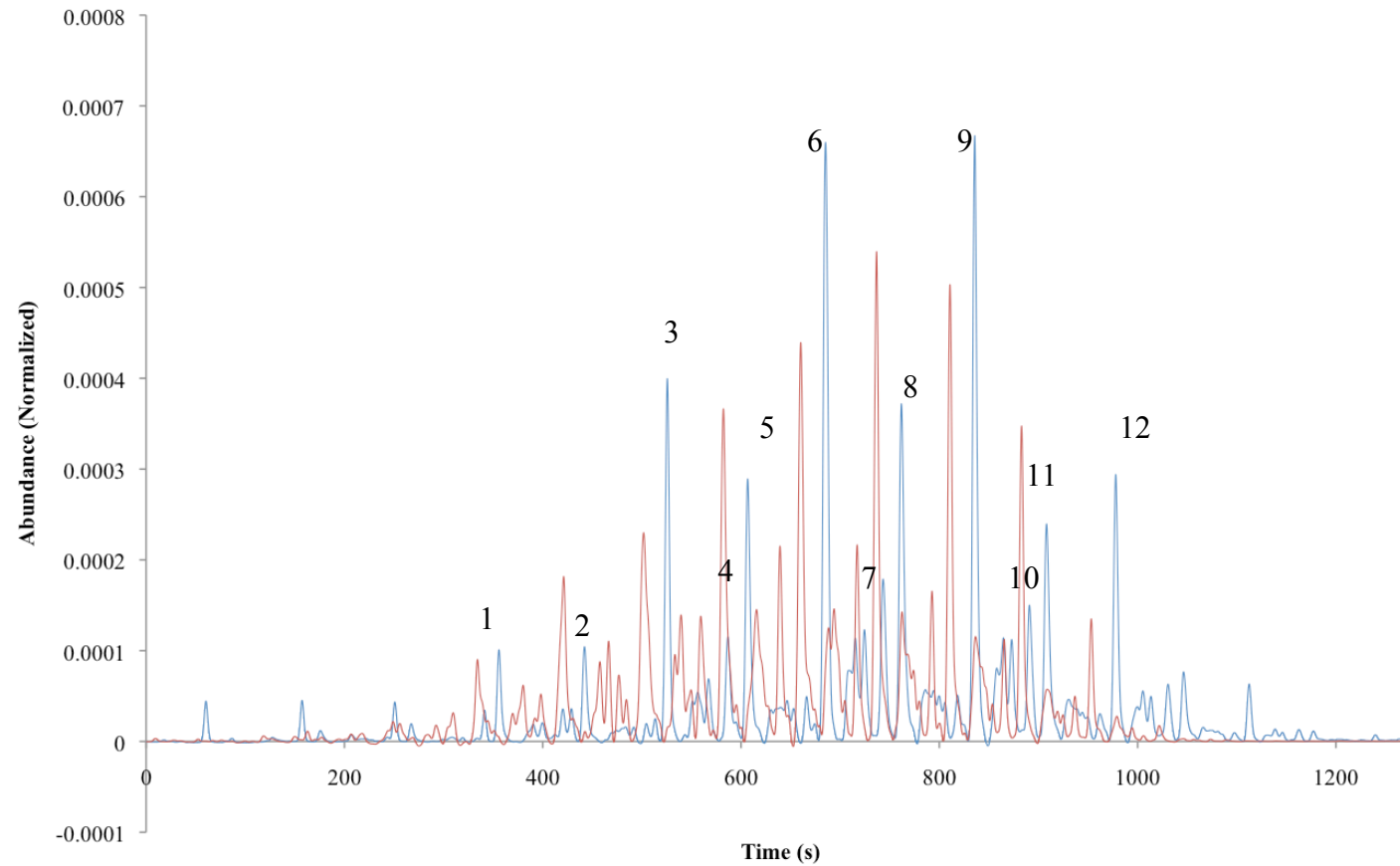




Figure 4.5. Mean gas chromatogram of the preen wax of breeding male bellbirds (red;  $n = 13$ ) and of adult shining cuckoos (blue;  $n = 10$ ).

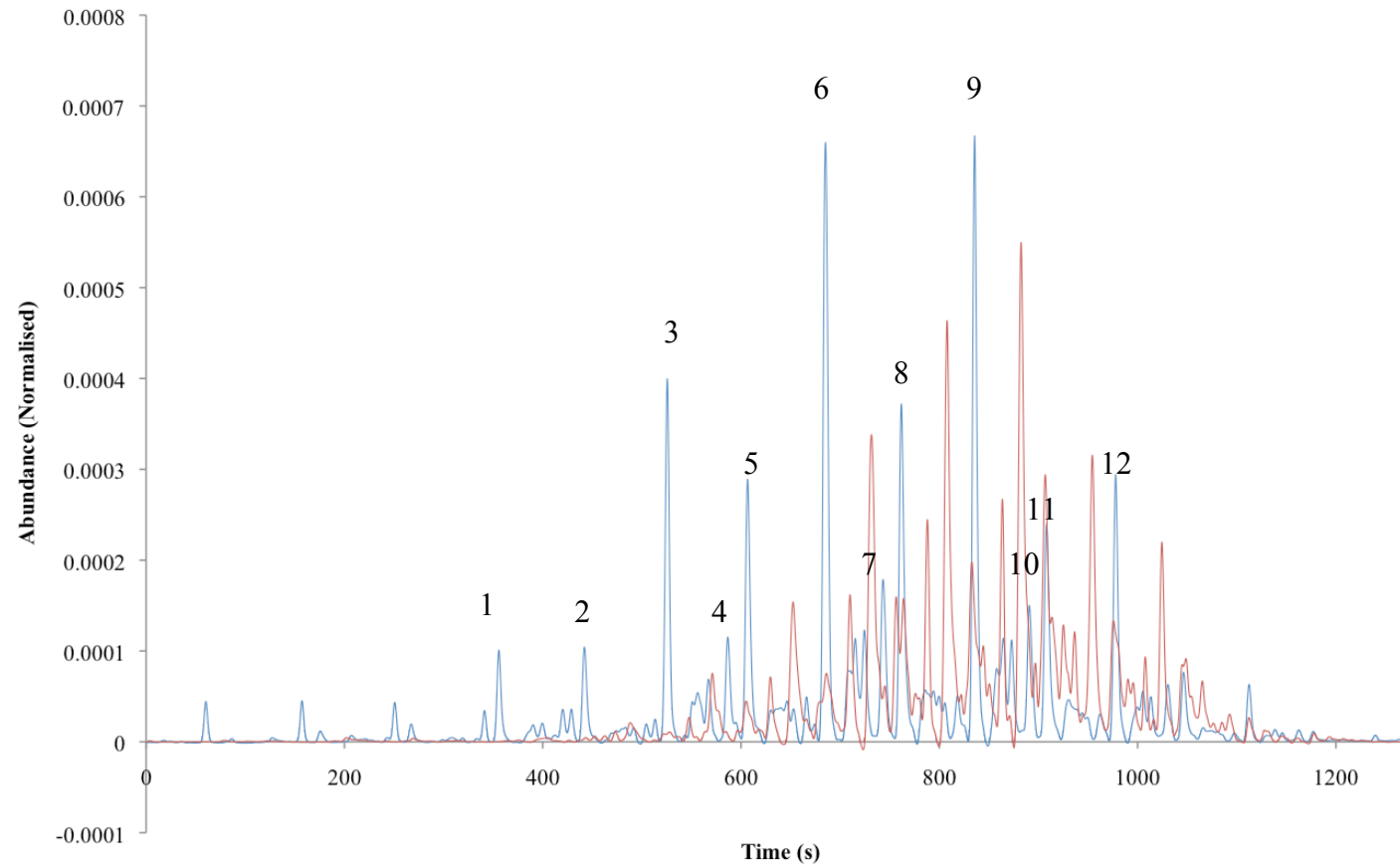


Figure 4.6. Mean gas chromatogram of the preen wax of breeding female bellbirds (red;  $n = 16$ ) and of adult shining cuckoos (blue;  $n = 10$ ).

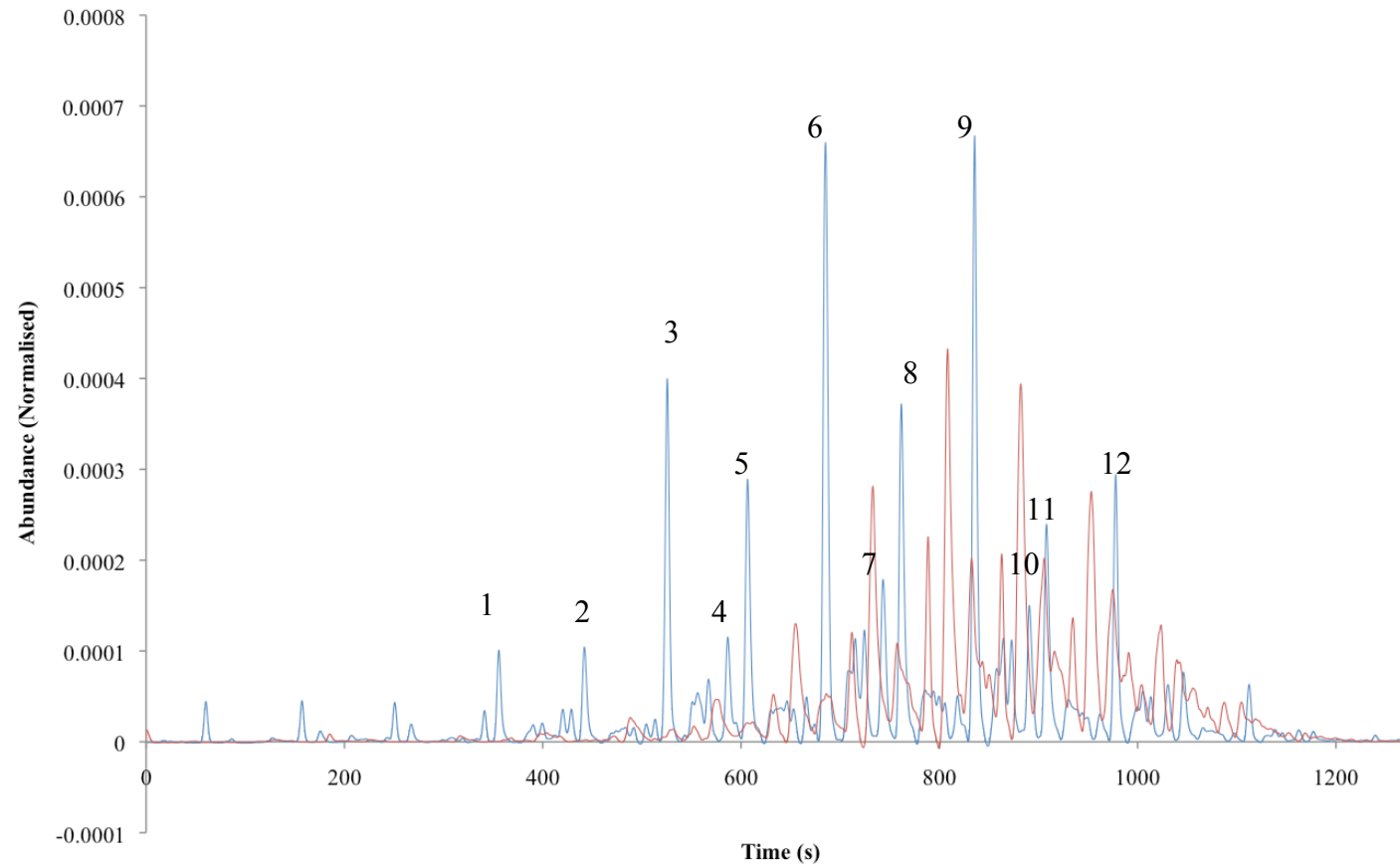


Figure 4.7. Mean gas chromatogram of the preen wax of breeding male fantails (red;  $n = 3$ ) and of adult shining cuckoos (blue;  $n = 10$ ).

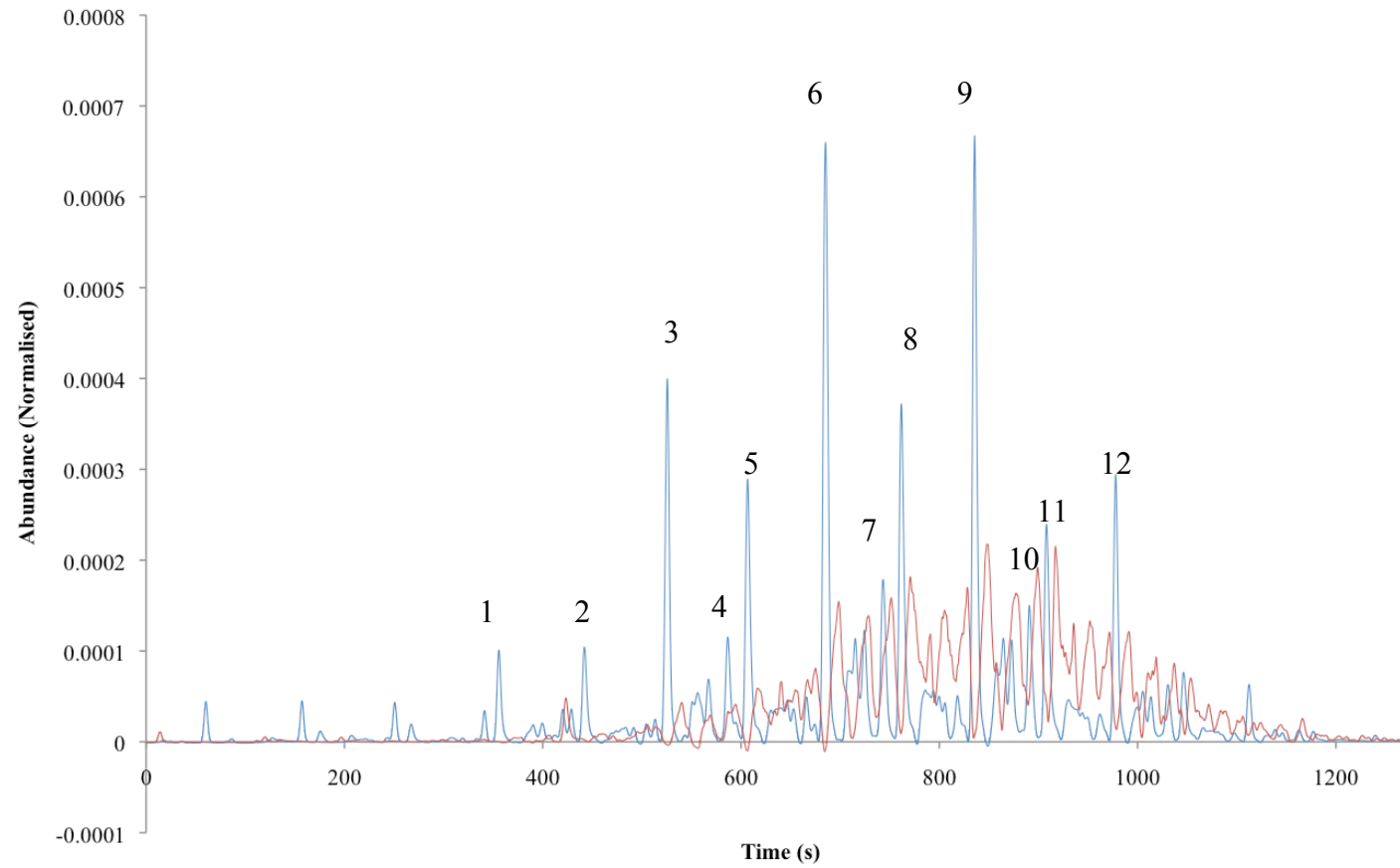


Figure 4.8. Mean gas chromatogram of the preen wax of breeding female fantails (red;  $n = 12$ ) and of adult shining cuckoos (blue;  $n = 10$ ).

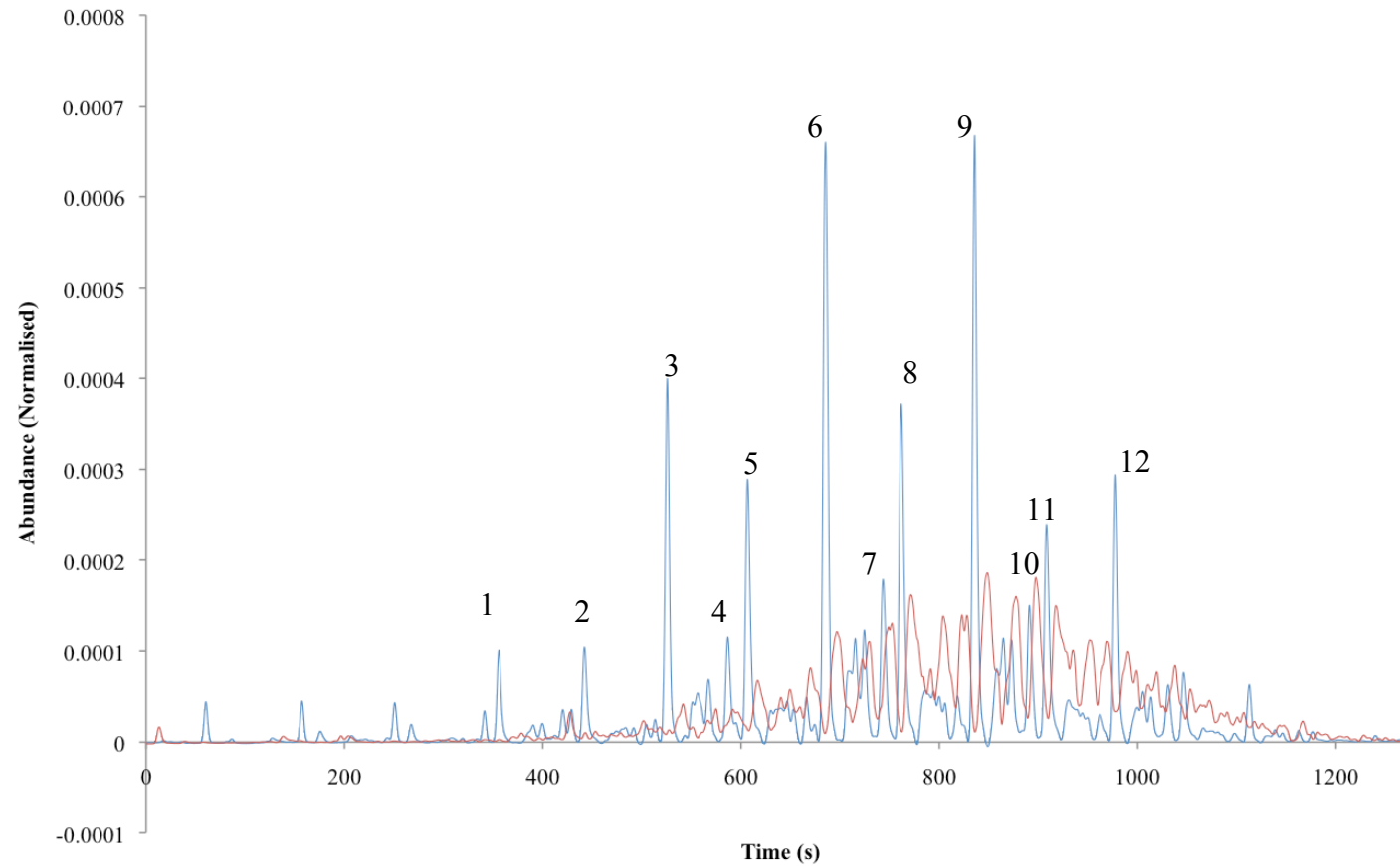


Figure 4.9. Mean gas chromatogram of the preen wax of breeding male New Zealand robins (red;  $n = 9$ ) and of adult shining cuckoos (blue;  $n = 10$ ).

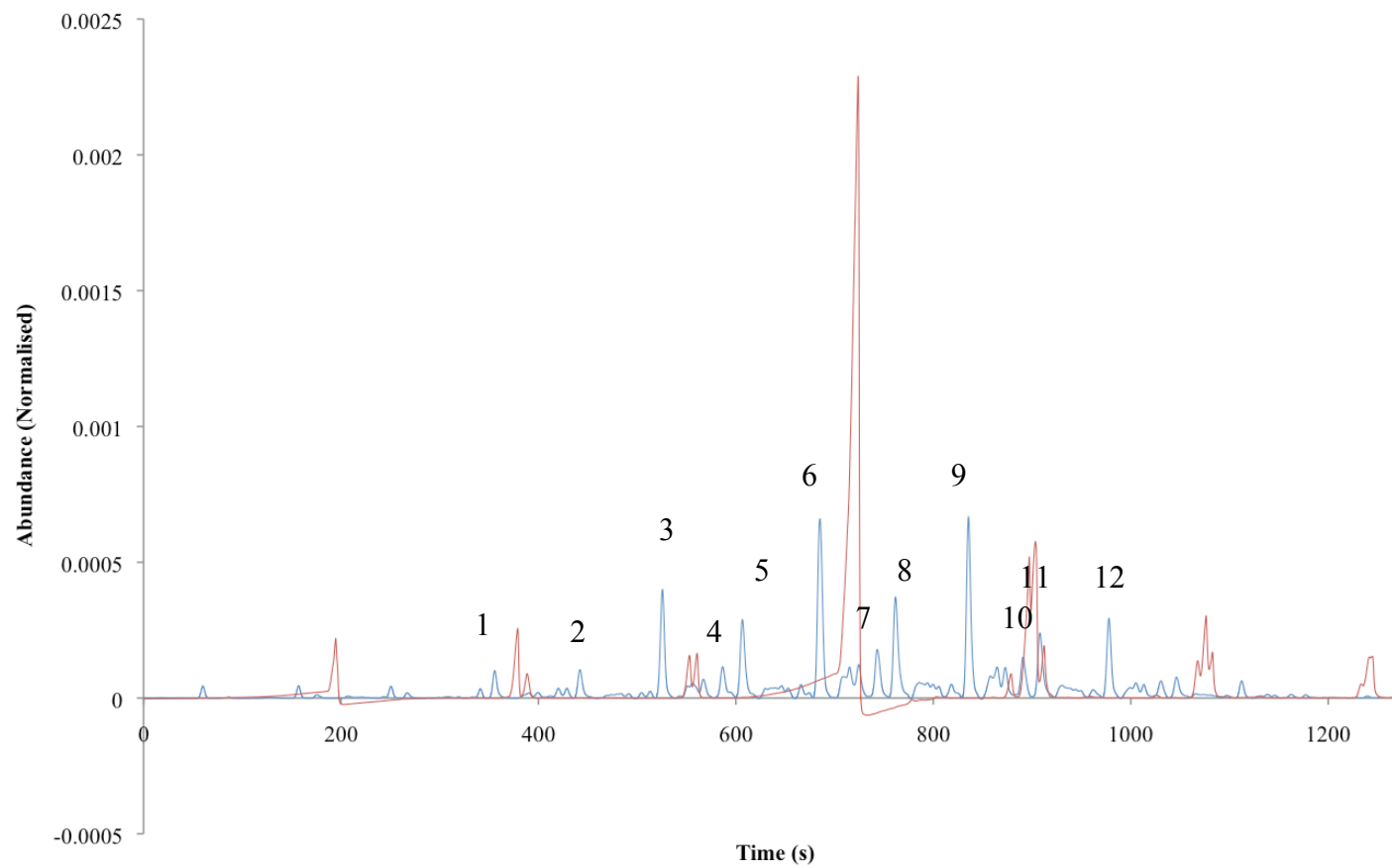


Figure 4.10. Mean gas chromatogram of the preen wax of breeding female New Zealand robins (red;  $n = 7$ ) and of adult shining cuckoos (blue;  $n = 10$ ).

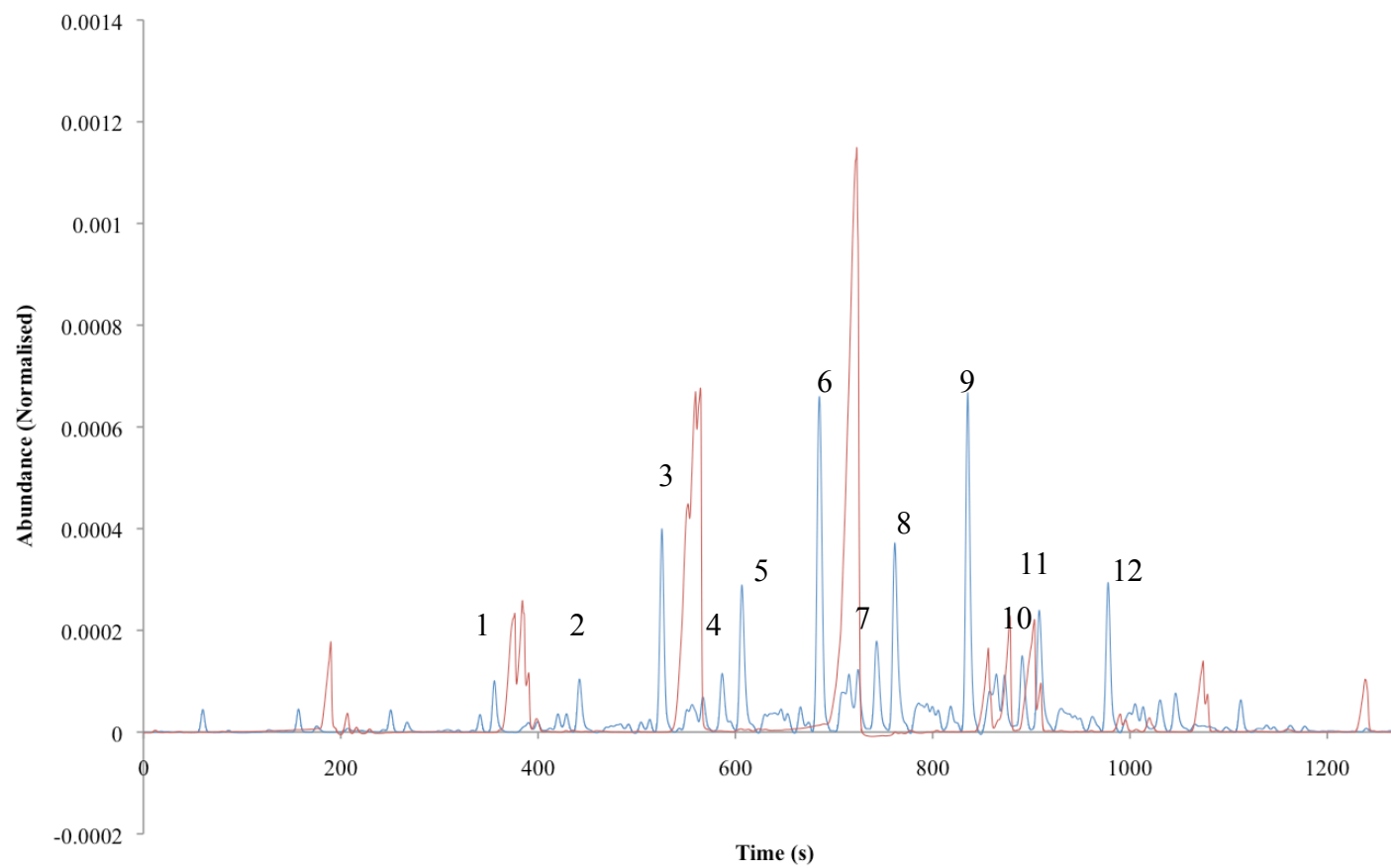


Figure 4.11. Mean gas chromatogram of the preen wax of breeding male rifleman (red;  $n = 4$ ) and of adult shining cuckoos (blue;  $n = 10$ ).

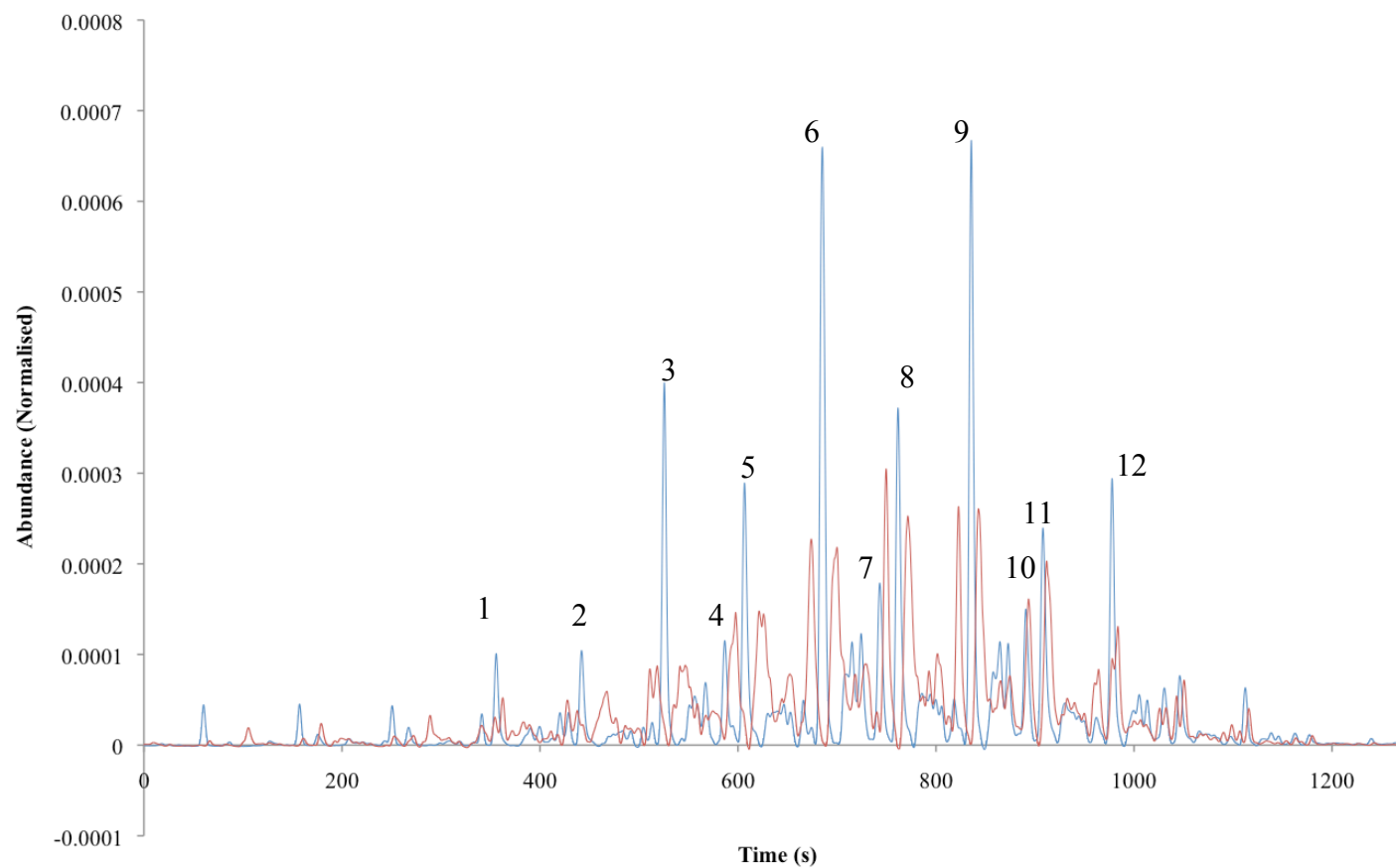


Figure 4.12. Mean gas chromatogram of the preen wax of breeding female rifleman (red;  $n = 4$ ) and of adult shining cuckoos (blue;  $n = 10$ ).

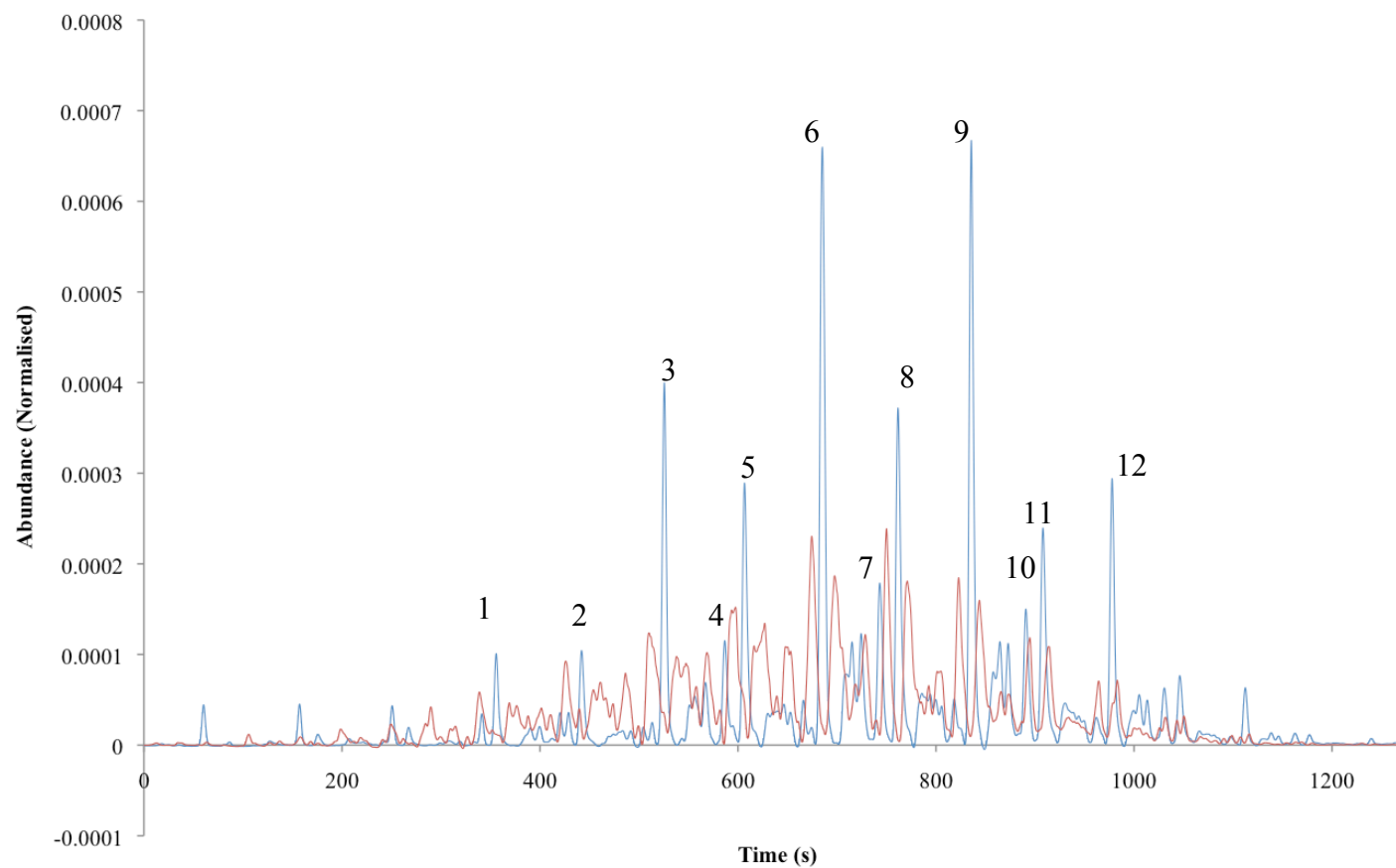




Figure 4.13. Mean gas chromatogram of the preen wax of breeding male brown creepers (red;  $n = 6$ ) and of adult shining cuckoos (blue;  $n = 10$ ).

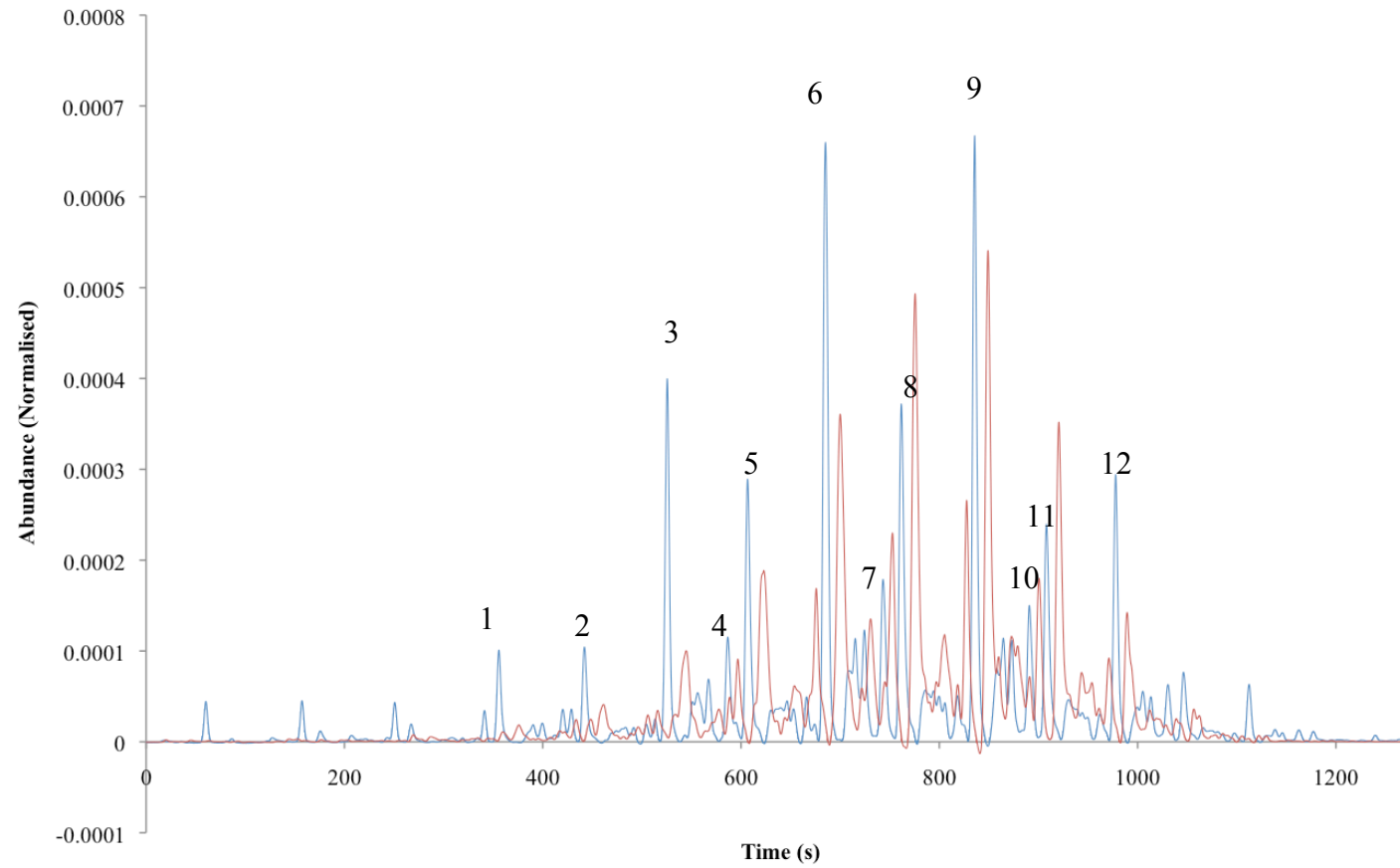


Figure 4.14. Mean gas chromatogram of the preen wax of breeding female brown creepers (red;  $n = 4$ ) and of adult shining cuckoos (blue;  $n = 10$ ).

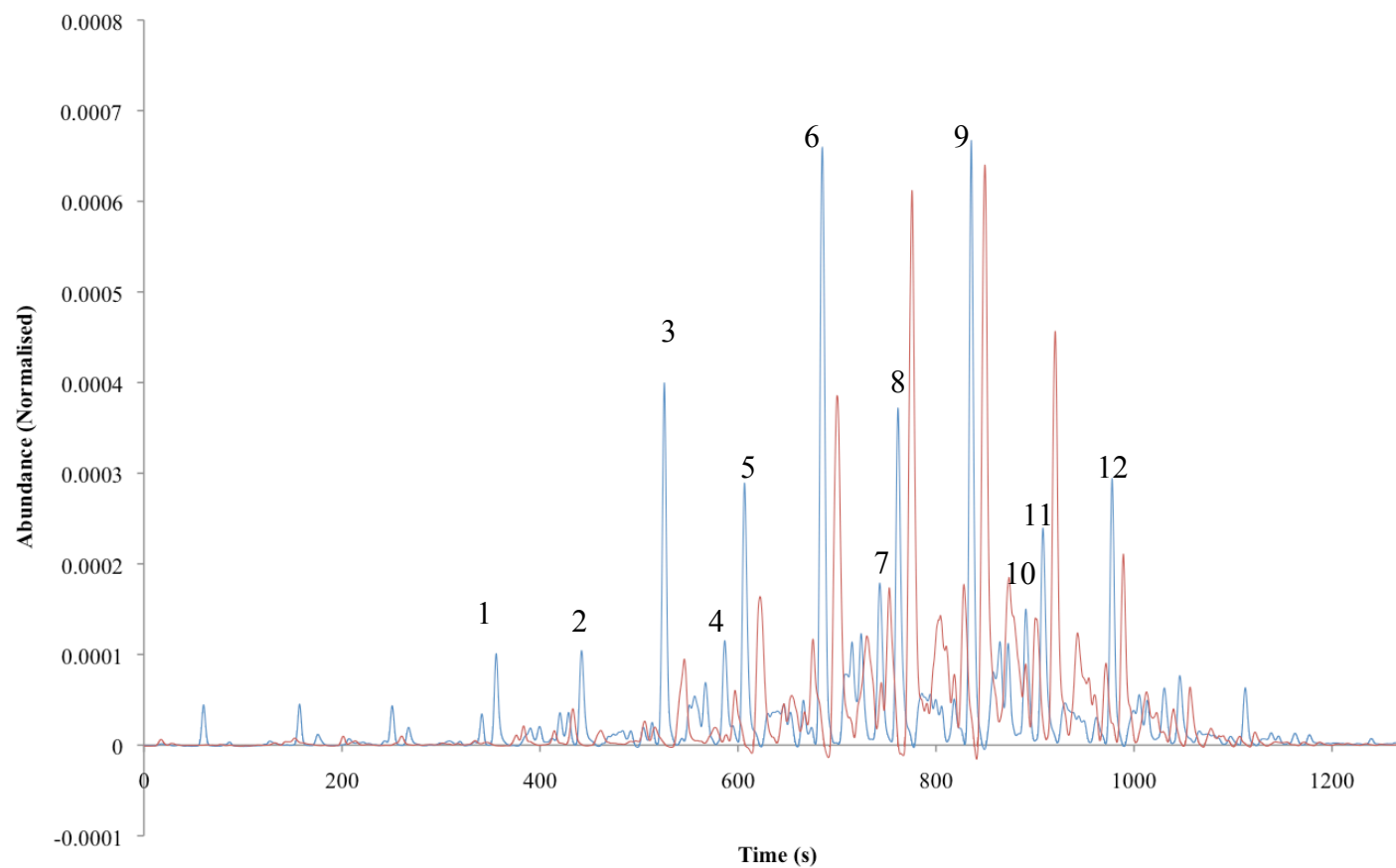


Figure 4.15. Mean gas chromatogram of the preen wax of breeding male silvereyes (red;  $n = 9$ ) and of adult shining cuckoos (blue;  $n = 10$ ).

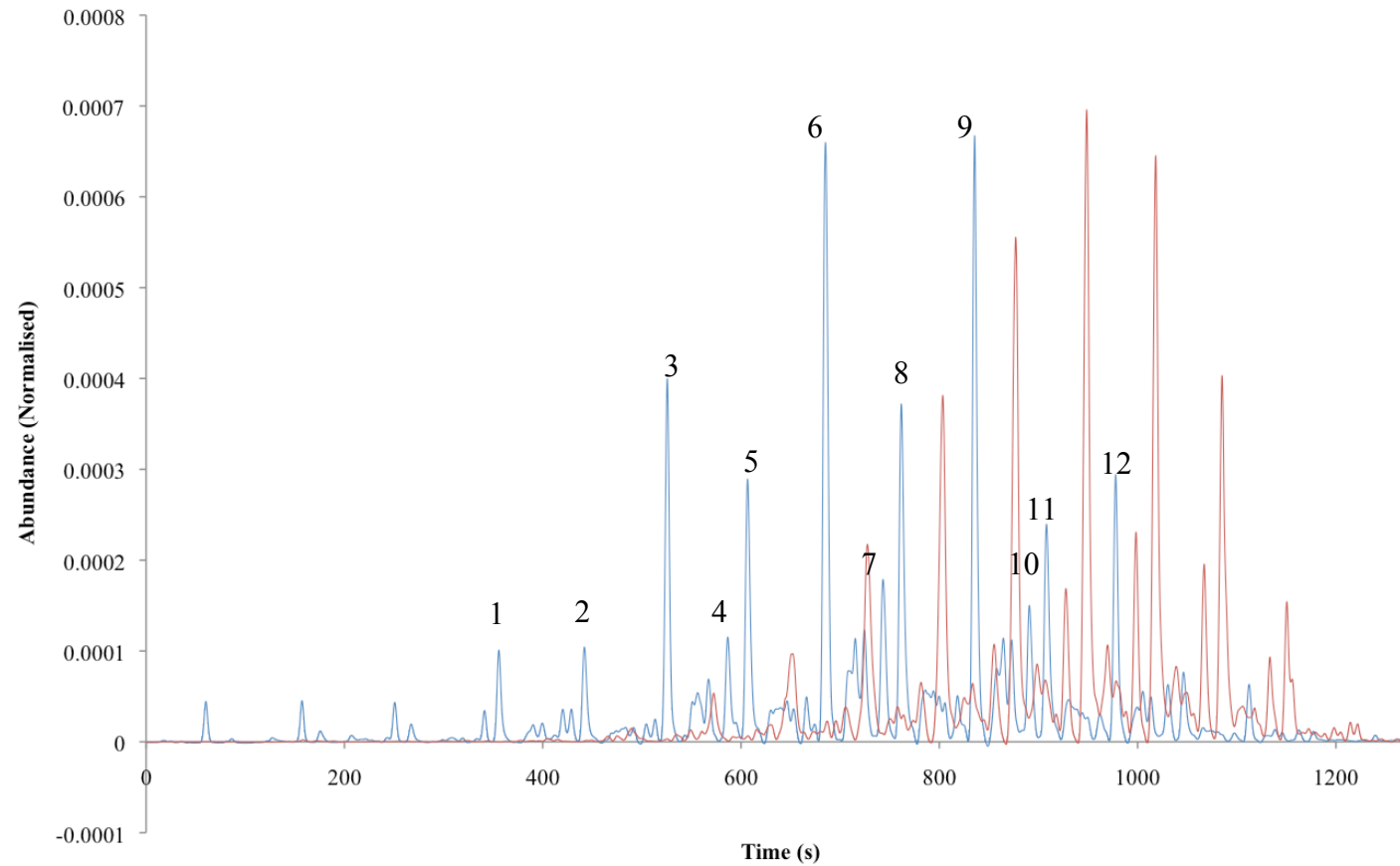


Figure 4.16. Mean gas chromatogram of the preen wax of breeding female silvereyes (red;  $n = 2$ ) and of adult shining cuckoos (blue;  $n = 10$ ).

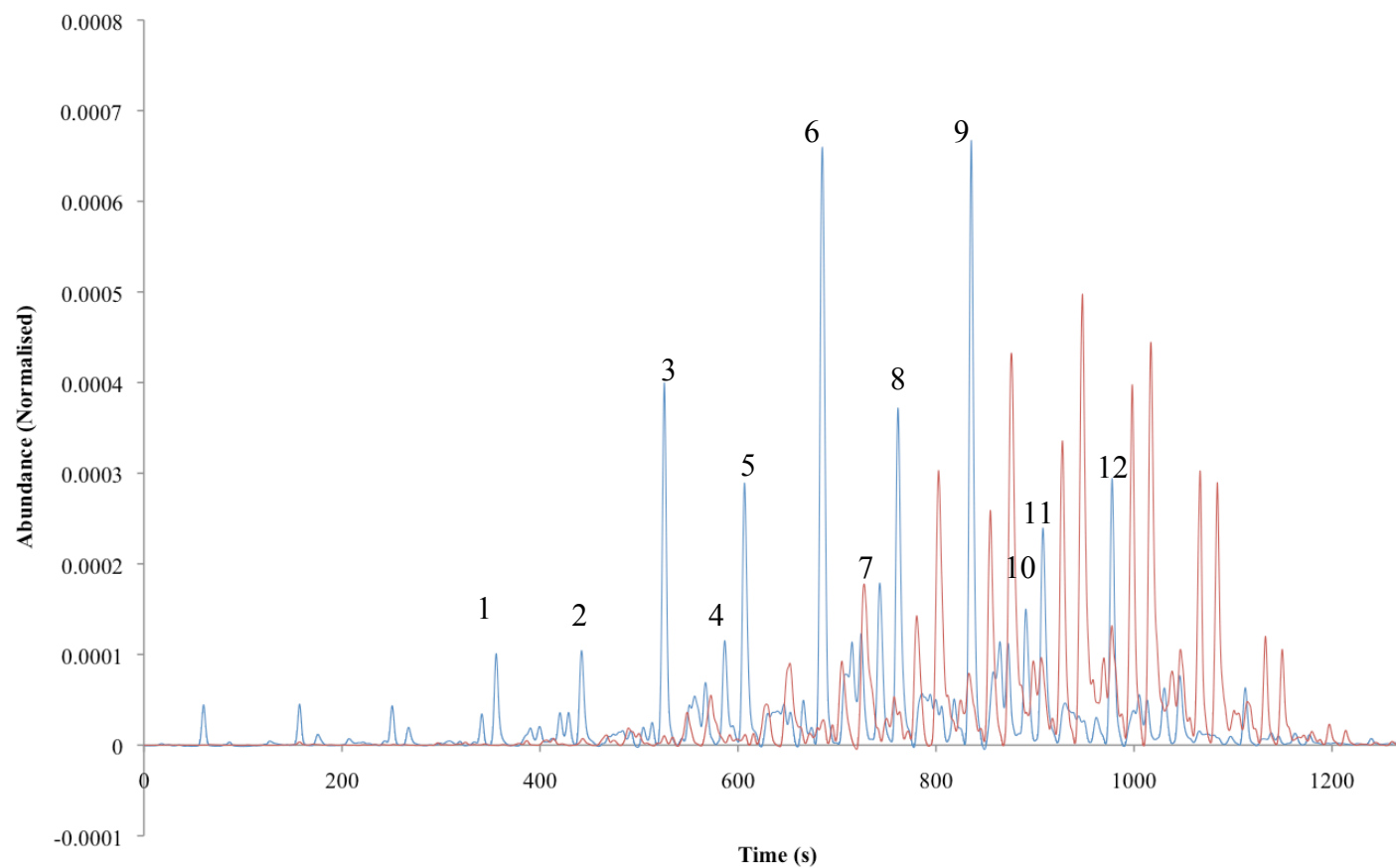


Figure 4.17. Mean gas chromatogram of the preen wax of breeding adult song thrush (red;  $n = 3$ ) and of adult shining cuckoos (blue;  $n = 10$ ).

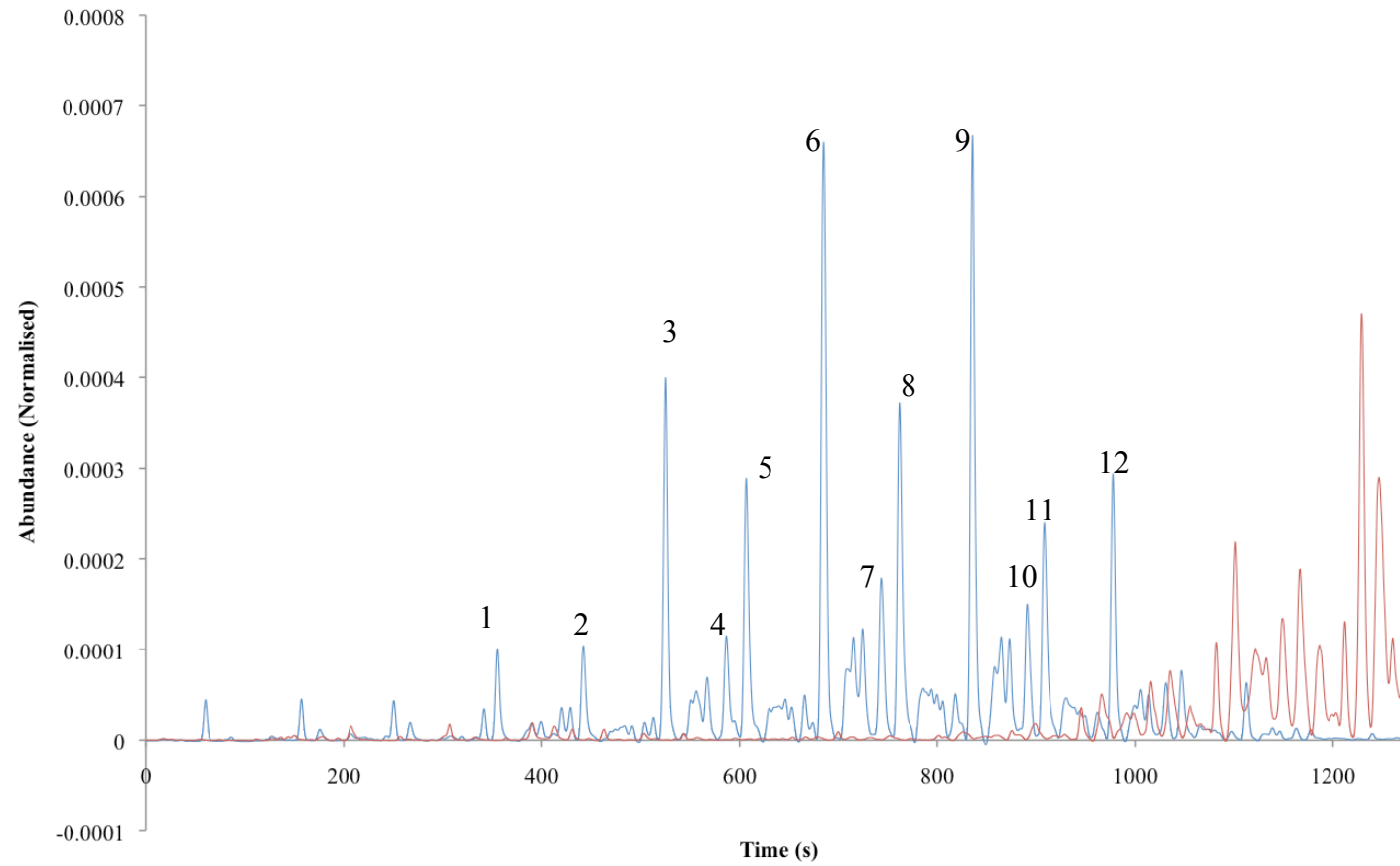


Figure 4.18. Mean gas chromatogram of the preen wax of grey warbler nestlings (red;  $n = 5$ ) and of shining cuckoo nestlings (blue;  $n = 3$ ).

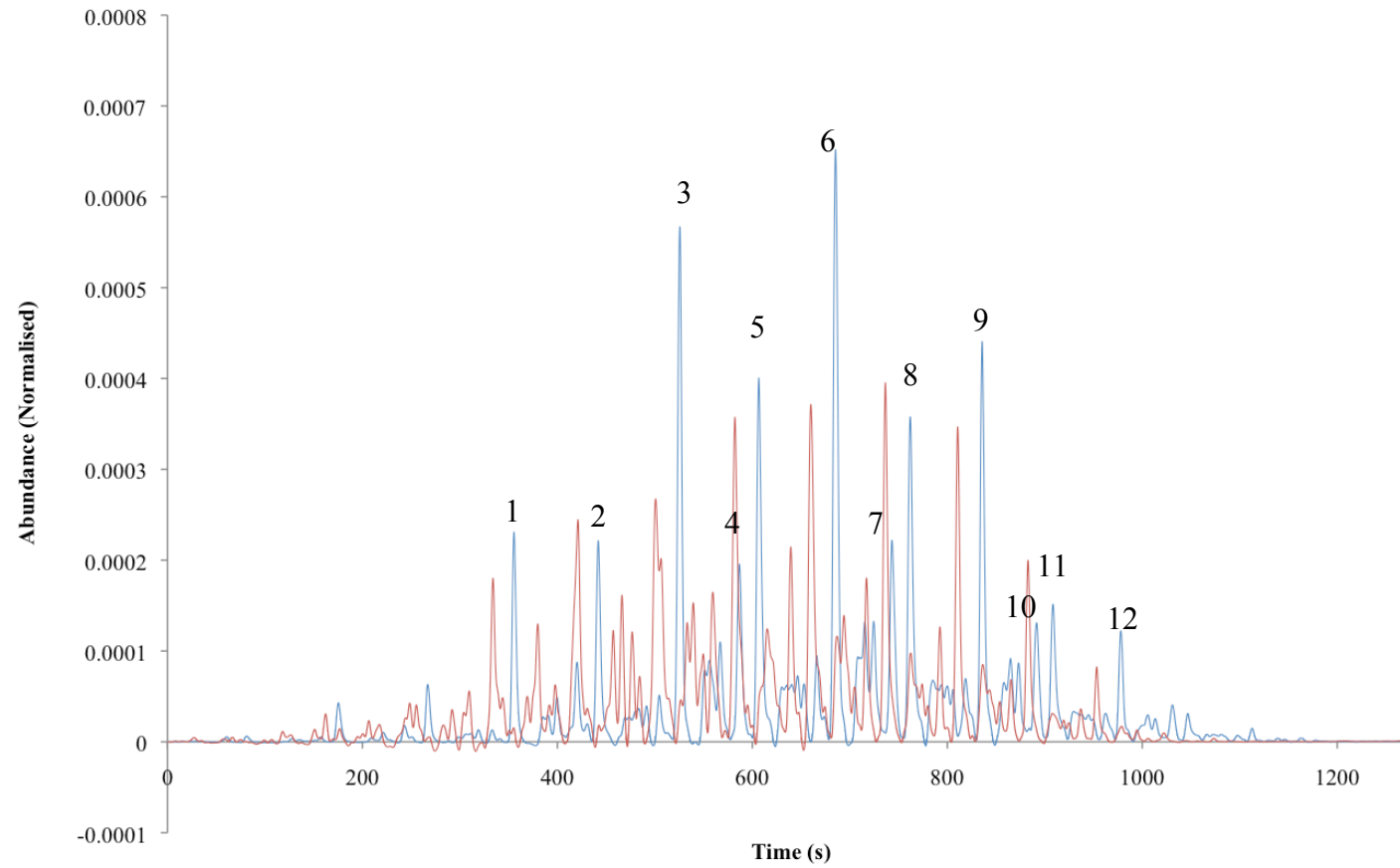


Figure 4.19. Mean gas chromatogram of the preen wax of fantail nestlings (red;  $n = 5$ ) and of shining cuckoo nestlings (blue;  $n = 3$ ).

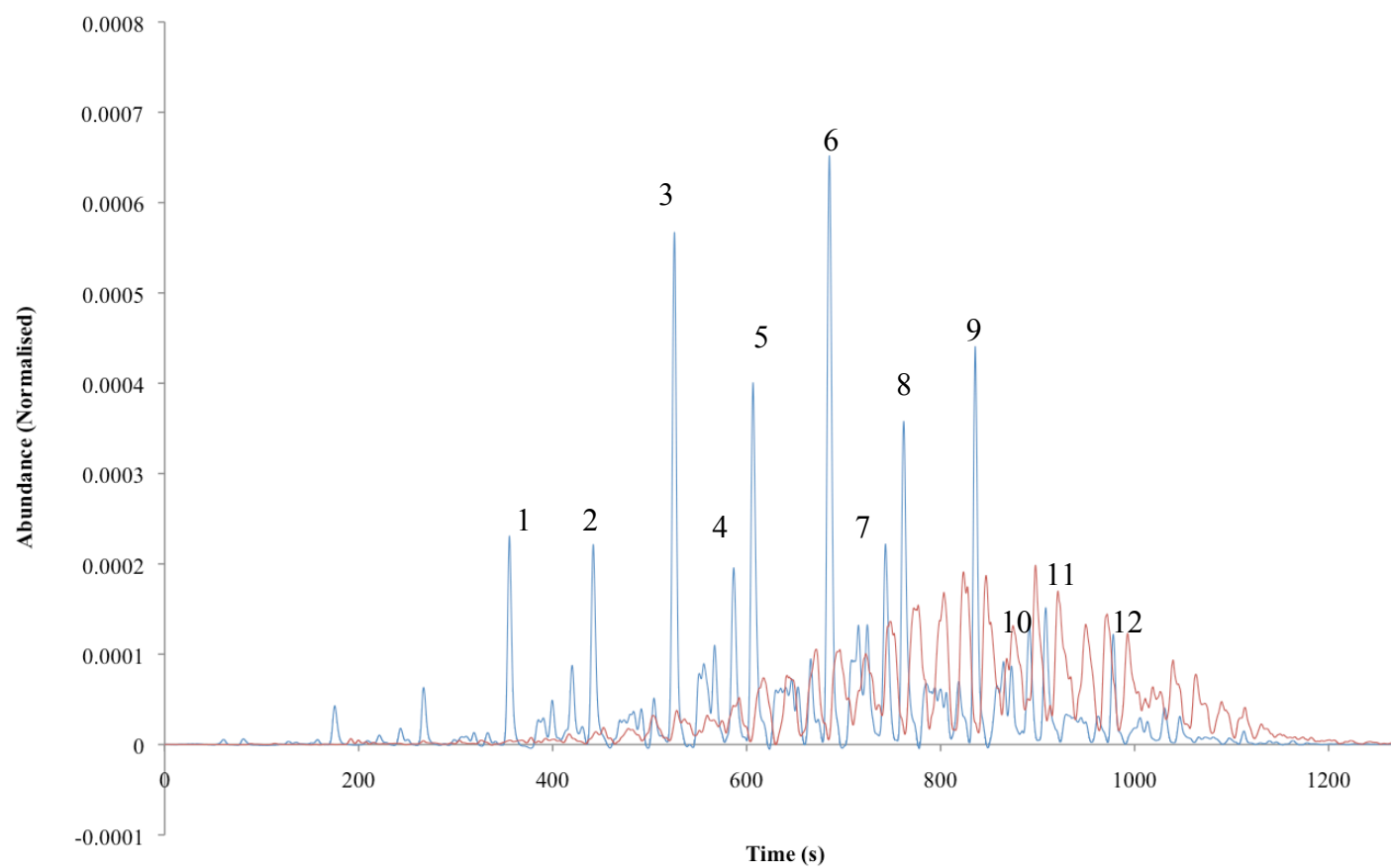


Figure 4.20. Mean gas chromatogram of the preen wax of New Zealand robin nestlings (red;  $n = 6$ ) and of shining cuckoo nestlings (blue;  $n = 3$ ).

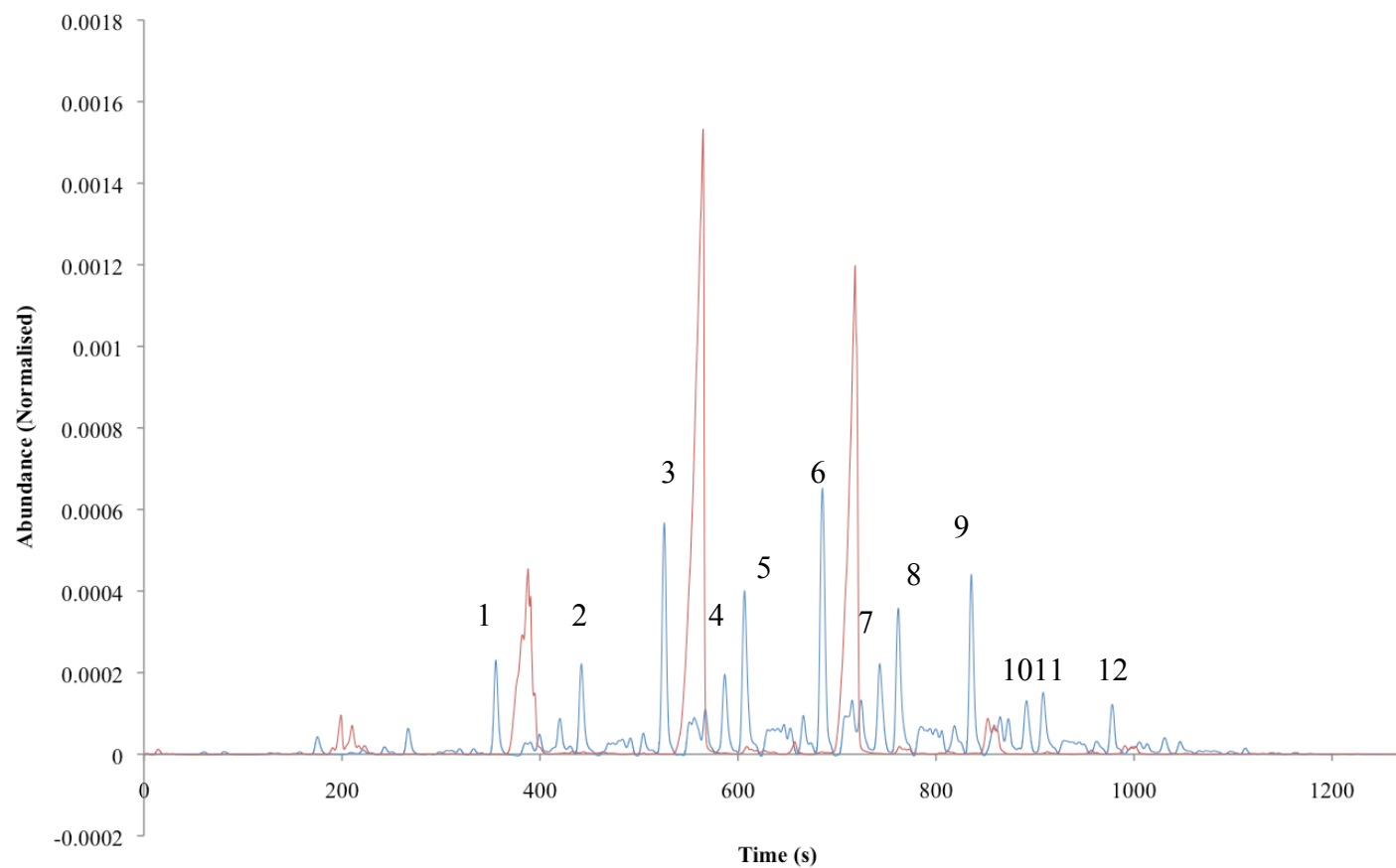
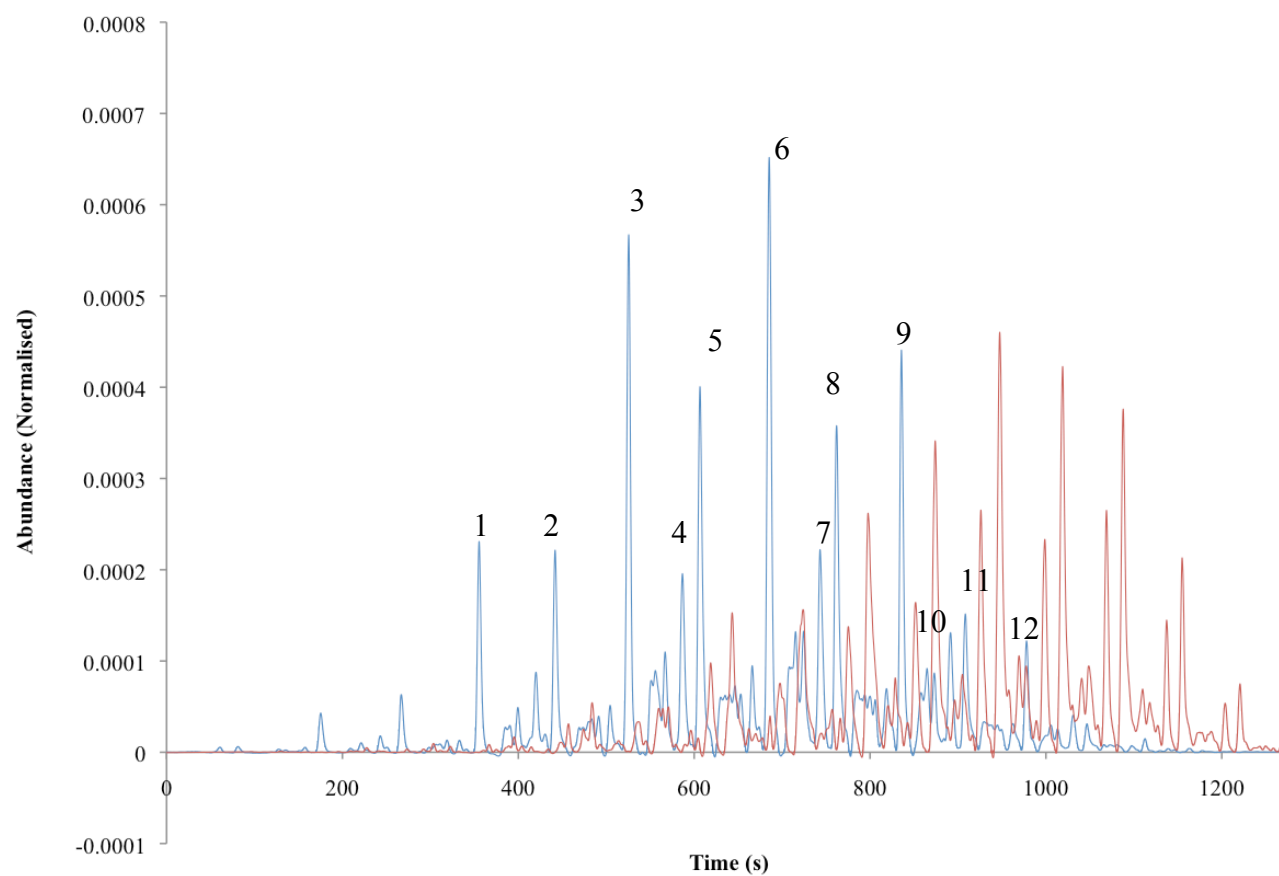




Figure 4.21. Mean gas chromatogram of the preen wax of a silvereye nestlings (red;  $n = 1$ ) and of shining cuckoo nestlings (blue;  $n = 3$ ).



## **Chapter 5: Does the incubation behaviour of song thrush *Turdus philomelos* change in response to the odours of conspecifics and heterospecifics?**

### ***5.1 Abstract***

Recent research has revealed the importance of olfaction to birds in many ecological contexts. However, the use of olfaction in the coevolutionary interactions between avian brood parasites and their hosts has never been investigated. Uropygial gland secretions (preen wax) are likely a key source of odour in birds because birds spread preen wax on their feathers and preen wax contains volatile compounds. Preen wax composition has been found to vary among species and between the sexes and thus preen wax odour may provide important information. In this study, I tested the hypothesis that song thrush *Turdus philomelos* use olfaction to assess the risk of parasitism. Song thrush reject the eggs of the common cuckoo *Cuculus canorus* and intraspecific parasites at intermediate and low frequencies, respectively. I measured the effect of applying the preen wax of conspecifics and of a non-parasitic species, the bellbird *Anthornis melanura*, to active song thrush nests on the incubation behaviour of female song thrush. Bellbirds are not parasitic, but their preen wax strongly differs in composition from that of song thrush (see below). I expected bellbird odour to elicit behavioural changes that might be associated with a heterospecific brood

parasite. If females use olfactory cues to detect brood parasitism, then I expected that the experimental addition of a foreign odour should lead to increased nest desertion or increased inspection of the nest contents. The incubation behaviour of song thrush did not change significantly within 3 h after the application of foreign preen wax or 24 h later. The results suggest song thrush do not respond to either the odours of conspecifics or bellbirds on the nest with changes in incubation behaviour.

## 5.2 Introduction

Recent research has revealed that olfactory-mediated communication may be as important for birds as it is for mammals (Roper 1999; Hagelin and Jones 2007; Balthazart and Taziaux 2009). Passerines, previously thought to be unlikely candidates for olfactory-mediated communication because of the small size of their olfactory bulb relative to the size of their brains (Bang and Cobb 1968), have recently been shown to possess fairly sophisticated olfactory capabilities. Genomic studies have shown that certain passerines, such as the blue tit *Cyanistes caeruleus*, have nearly as many functional olfactory receptor genes as the snow petrel *Pagodroma nivea*, a Procellariiform (Steiger et al. 2008; Steiger et al. 2009), which has one of the largest reported olfactory bulb to brain ratios among birds (Bang and Cobb 1968). Additionally, behavioural studies have demonstrated that some passerines use olfaction in kin recognition (Krause et al. 2012), sex recognition (Whittaker et al. 2011; Amo et al. 2012), nest identification (Caspers and Krause 2011), recognition of offspring (Cohen 1981), detection of visits by conspecifics and heterospecifics at the

nest (Whittaker et al. 2009) and predator detection (Amo et al. 2008; Roth et al. 2008 but see Godard et al. 2007).

Despite the recent impetus of research in chemical communication in birds, few studies have investigated the role of olfaction or controlled for odour in the interactions between avian brood parasites and their hosts. In terms of detecting foreign eggs and nestlings, visual cues are likely the most important sensory cue for the coevolutionary arms race and it has led to remarkable egg and nestling mimicry seen between many hosts and parasites (Stokke et al. 1999; Cherry and Bennett 2001; Payne 2005; Avilés et al. 2006; Cherry et al. 2007a, b; Honza et al. 2007; Moskát et al. 2008; Underwood and Sealy 2008; Soler 2009; Avilés et al. 2010). However, the evolutionary arms race may continue with, or in conjunction with, odour mimicry when visual mimicry by brood parasites increases the cost to hosts of detecting the eggs and nestlings of conspecifics that are already very similar in appearance. One of the key sources of odour in birds is from secretions of the uropygial gland (hereafter preen wax).

Most bird species possess uropygial glands that secrete preen wax which is then spread on the feathers while preening. Preen wax is posited to function in waterproofing (Elder 1954; Fabricius 1959; Stettenheim 1972; Elowson 1984; Sweeney et al. 2004), altering or enhancing the appearance of feathers (Piersma et al. 1999; Delhey et al. 2007; Piau et al. 2008), repelling or killing feather-degrading ectoparasites, predators, and mosquitoes (Hwang et al. 1980; Kramer et al. 1980; Hwang et al. 1982; Jacob and Ziswiler 1982; Hwang et al. 1984; Jacob et al. 1997; Douglas et al. 2001; Moyer et al. 2003; Burger et al. 2004; Douglas 2004), and preventing microbial and fungal growth (Martín-Vivaldi et al. 2010).

The volatile compounds found in preen wax may also function as chemical odorants in olfactory-mediated communication because they vary among species (Jacob and Ziswiler 1982; Sweeney et al. 2004; Haribal et al. 2005; Haribal et al. 2009; Whittaker et al. 2009), populations (Whittaker et al. 2010) and individuals (De Leon et al. 2003; Bonadonna and Nevitt 2004; Bonadonna et al. 2007; Caro and Balthazart 2010; Karlsson et al. 2010; Whittaker et al. 2010). In addition, preen wax composition varies according to the reproductive period and with age (Kolattukudy and Sawaya 1974; Piersma et al. 1999; Soini et al. 2007; Whelan et al. 2010; Whittaker et al. 2010; Amo et al. 2012), and differs between the sexes in some species (Zhang et al. 2009; Whittaker et al. 2010; Zhang et al. 2010; Amo et al. 2012). Furthermore, these odours have been found to be repeatable (Bonadonna et al. 2007; Whittaker et al. 2010) and important in intraspecific communication in seabirds (Hagelin et al. 2003; Bonadonna and Nevitt 2004; Bonadonna et al. 2007; Hagelin 2007), penguins (Coffin et al. 2011), chickens (Hirao et al. 2009), and certain passerines (Whittaker et al. 2009; Zhang et al. 2010; Amo et al. 2012).

Since preen wax odours have been shown to contain important socio-ecological information and since the use of this information has been demonstrated in some species, it is plausible that preen wax odours are used by hosts when discriminating against brood parasites or assessing the risk of parasitism. Whittaker et al. (2009) experimentally determined that dark-eyed juncos *Junco hyemalis* could detect and discriminate between the preen wax of conspecifics, heterospecifics (i.e., mockingbird *Mimus polyglottos*), and their own preen wax when applied to the nest. Although this study did not investigate brood parasitism specifically, it showed that passerines may gain information about visitors at their nest from olfactory cues. However, the response of dark-eyed juncos (i.e., change in length of first incubation

bout) to the presence of foreign preen wax odours at the nest was subtle (Whittaker et al. 2009). Larger reactions, such as egg ejection or desertion, were not expected since the dark-eyed junco does not reject brood parasitism by brown-headed cowbirds *Molothrus ater* (Whittaker et al. 2009). However, it is possible that hosts that reject brood parasitism may have larger reactions to nest odour manipulations.

In this study, I tested the hypothesis that hosts use odour at the nest to assess the risk of parasitism by brood parasites. Adaptive changes in behaviour at the nest in response to nest odour manipulations have been recorded in other species (Amo et al. 2008; Mennerat 2008; Whittaker et al. 2009). To test this hypothesis, I manipulated the odour of song thrush *Turdus philomelos* nests and measured the response of the female nest owners in terms of changes in incubation behaviour between control treatments and one of two odour treatments: (1) conspecific preen wax or, (2) bellbird *Anthornis melanura* preen wax. Song thrush discriminate against the eggs of common cuckoos *Cuculus canorus* and conspecifics at intermediate and low frequencies, respectively (Hale and Briskie 2007; Honza et al. 2007; Samas et al. 2011) by grasping them with their bill and removing them from the nest (Moksnes et al. 1991; Honza et al. 2007). Bellbirds are not parasitic, but because their preen wax strongly differs in composition from that of song thrush (see below), I expected bellbird odour to elicit behavioural changes that might be associated with a heterospecific brood parasite. I predicted that if females use odour cues to detect parasitism (either conspecific or heterospecific), they would inspect their clutches more often and for longer periods of time when nest odour was manipulated.

### 5.3 Methods

#### *Effect of foreign preen wax odour on song thrush incubation behaviour*

I tested the effect of foreign preen wax on incubation behaviour in an introduced song thrush population in Kowhai Bush (42°23' S, 173°37' E), a 240 ha block of forest located ~10 km from Kaikoura, South Island, New Zealand from 17 October to 3 December 2012. The site was described in detail in Gill (1980). Song thrush were introduced to New Zealand from Britain in the mid-19<sup>th</sup> century (Thomson 1922) but despite being separated from the common cuckoo for ~ 130 years they retain the ability to discriminate and eject common cuckoo eggs and the eggs of conspecifics at intermediate frequencies (Hale and Briskie 2007). There are no reliable records of brood parasitism of song thrush by native parasitic cuckoos in New Zealand (Gill 1983; Higgins and Peter 2002; Higgins et al. 2006). Nest building and incubation is performed by the female only (Congdon 2010), thus the majority of the chemical odorants from preen wax on the nest are likely from the female parent song thrush. Males do not incubate (Congdon 2010).

#### *Experimental protocol*

I video recorded the incubation behaviour of song thrush at nests that were treated with either conspecific or bellbird preen wax. Each nest was subjected to three treatments (i.e, 2 controls and 1 preen wax treatment) that were administered in the same order at each nest on different but subsequent days. A within-subject design was used and is preferable in this experiment because it eliminates individual variation because individuals acted as their own controls (Gravetter and Forzano 2003). However, within-subject designs also have disadvantages such as the introduction of carryover effects, habituation, and environmental events (Gravetter and Forzano

2003). I tried to control for carry-over effects by waiting approximately 24 h between trials and, for habituation and environmental events by having a control trial before and after the treatment trial. On the first day, I added 2–3 drops of water to the nest and recorded the response of the female song thrush (control trial 1). On the second day, I treated the nest with  $\sim 0.4 \mu\text{l}$  of preen wax (range:  $0.1\text{--}0.8 \mu\text{l}$ ) from a conspecific or a bellbird and recorded the response of the female song thrush (preen wax trial). The amount of preen wax is based on the ‘length’ of the sample in a  $100 \mu\text{l}$  of a glass capillary tube divided by the length of the tube and multiplied by the volume of the tube. This method is approximate but it provided a rough estimate of the preen wax applied to each nest which, in this study, is likely more than is transferred from the feathers of a visiting brood parasite during a parasitism event. On the third day, I treated the nest with 2–3 drops of water again and recorded the response of the female song thrush (control trial 2). Samples (i.e., preen wax or water) were added to the nest lining under the eggs in order to change the overall odour of the nest and to avoid providing any visual cues (i.e., the water or preen wax sample was hidden from view so only olfactory cues were available to the host). Control (water) and preen wax samples were stored in  $100 \mu\text{l}$  glass capillary tubes sealed with Parafilm® wax, labelled and kept at  $-20^{\circ}\text{C}$  until required in trials. All trials were initiated from early to mid incubation. Trials began as early as 8.05 min after sunrise and as late as 155.68 min after sunrise (mean =  $72.48 \pm 4.50$  min,  $n = 57$ ) but the start times among trials at individual nests differed by only  $33.73 \pm 4.79$  min (range: 10.93–96.07 min,  $n = 19$ ).

Nests were video recorded for 5 to 6 hours immediately following the application of the treatment to the nest. Because of the dense vegetation that surrounded most nests in Kowhai Bush, this distance was typically  $\sim 5$  m. Cameras



were removed after each recording session. Nests were recorded with Sony® DCR cameras on tripods that were covered with cryptically coloured cloth. It is not known how the subjects perceived the camera apparatus. However, any effect the camera apparatus may have had on the subjects was controlled by using camera apparatuses with similar appearances at each nest and for each trial.

The eggs and nests used in experiments were never touched. New latex gloves were worn when applying preen wax or water to nests. I blew on one end of the capillary tube to push the preen wax and water out of the tube. To control for the effect of my personal odour, I brushed my teeth with the same toothpaste every morning and used the same type and amount of sunscreen (“non-scented”) on my face to keep my odour constant across trials. I also wore dark shirts or jackets and the same hat every day to ensure that my appearance did not affect the behaviour of the subjects.

The first three hours after the initial return of a song thrush to the nest (observation period) were used for analysis. The time from when the researcher left the nest area and the initial return of a song thrush to the nest (latency) was recorded for every trial. Latency was not expected to differ with odour treatment as the odour cue is likely not detectable until the bird returns to the nest. The time that at least one song thrush was present on the nest, number of peers (i.e., visual inspection of the nest contents) and probes (i.e., touching of nest contents with bill) per hour on the nest, percentage of time spent peering and probing per hour on the nest, and length of the first visit to the nest were recorded to assess changes in incubation behaviour between trials.

In total, I video recorded 39 song thrush nests of which 19 were included in analyses. The sample sizes obtained in this study were sufficient to detect significant

changes in incubation behaviour in other studies (Whittaker et al. 2009). I analysed 171 h of video of incubation behaviour recorded at these 19 nests. Nests where a song thrush did not return to the nest (5 of 20 nests) or the female did not return to incubate the eggs (8 of 20 nests) within 3 h of the application of the treatment were not included in the analysis and remaining treatment trials were not recorded at these nests. Of the five nests where a song thrush did not return to the nest during a treatment trial, a song thrush did not return to the nest during the first control treatment trial at four nests and during the preen wax treatment trial at one nest. Of the eight nests where a song thrush returned to the nest but did not incubate, a song thrush did not return to incubate during the first control treatment trial at four nests, during the preen wax treatment trial at three nests, and during the second control treatment trial at one nest. However, at least one song thrush visited the nest during these treatment trials for an average of  $89.38 \pm 23.97$  s during an average of  $1.5 \pm 0.27$  visits. Additionally, nests were not included in the analysis if it rained during a trial (2 of 20 nests), if the eggs hatched (1 of 20 nests), if the nest was depredated between treatment trials (1 of 20 nests), if I was unable to film at a nest because of logistical constraints (1 of 20 nests), or if vegetation obscured the view of the nest in a way that prevented clear measurements of behaviour during the observation period (2 of 20 nests) in one or more of the treatment trials.

Preen wax samples used in trials were collected between 0730 and 1205 NZST from 26 September and 30 November 2012. Twelve of the 19 nests were tested with preen wax from 11 female and 1 male song thrush in breeding condition. The other seven nests were tested with preen wax from 6 female and 1 male bellbirds in breeding condition.

### *Statistical analyses*

The data were not normally distributed even after transformation and thus within-subject differences were analysed with Friedman two-way analysis of variance (ANOVA) by ranks (Daniel 1999). This test is the non-parametric version of the parametric ANOVA (Daniel 1999). All tests with p-values  $\leq 0.05$  were considered significant. All analyses were computed in STATISTICA 12 (Statsoft, Inc., Tulsa, OK, USA). All values are presented as the mean  $\pm$  SE.

### *Collection of preen wax samples*

Preen wax from adult bellbirds and song thrush was collected in and near Kowhai Bush (42°23' S, 173°37' E) and Waimangarara Bush (42°20' S, 173°40' E) located ~ 10 km from Kaikoura, New Zealand. Birds were captured with mist nests and banded with uniquely numbered Department of Conservation (New Zealand) bands. Female song thrush were also fitted with unique combinations of colour bands to be able to identify them later at nests to prevent using a song thrush's preen wax on her own nest. Preen wax samples were obtained by gently massaging the uropygial gland of captured birds with wax-tipped forceps until a small drop of preen wax was released (Soini et al. 2007). Preen wax that was going to be used in song thrush nest odour manipulations was collected and stored in sterile 100  $\mu$ l glass capillary tubes sealed with Parafilm® wax whereas samples used in chemical analyses were collected on inoculation loops and stored in glass vials (described in Chapter 4). Samples were kept on ice immediately after collection and transferred to a freezer at the end of the field day. Samples were stored at ~ -20°C (Soini et al. 2007; Whittaker et al. 2009) until required in experiments. Preen wax samples were removed from the freezer

approximately 12 h prior to their use in experiments. Each preen wax sample used in this study came from a different bird.

### *Chemical analysis*

The procedures I followed to collect and analyse the composition of preen wax are described in detail in Chapter 4.

## **5.4 Results**

### *Effect of foreign preen wax odour on incubation behaviour*

The data do not suggest incubation behaviour varied significantly among control and preen wax trials for all of the behavioural variables I measured (Table 5.1). There was no trend for behavioural change among individuals between any of the trials for any of the measured variables. Song thrush almost invariably settled on their clutches upon first return to the nest after the application of the preen wax or water treatment except during the control 1 and control 2 trial at one nest tested with bellbird preen wax and during the control 1 trial at another nest tested with bellbird preen wax, where a song thrush only settled on the nest after it returned to the nest for a second time. Also, song thrush almost invariably settled on the nest without first probing the contents upon their first return except at three nests where song thrush probed the nest contents once before settling on the nest for the first time during two control 2 trials and one preen wax (bellbird) trial. Song thrush returned to the nest following the application of preen wax or water in a mean of  $665.7 \pm 139.4$ ,  $463.2 \pm 145.5$  and  $666.7 \pm 303.8$  s for control trial 1, preen wax trial and control trial 2, respectively.

### *Preen wax odour variability*

Gas chromatography analyses of song thrush and bellbird preen wax suggests their compositions differ according to species (Fig. 5.1). Mean retention time for the 10 largest peaks of the gas chromatography trace of bellbird preen wax was  $879.3 \pm 28.7$  s (range: 734.5–1027.3 s;  $n = 29$ ) whereas mean retention time for the 10 largest peaks of song thrush preen wax was  $1253.6 \pm 29.4$  s (range: 1104.6–1372.1;  $n = 3$ , 2 males and 1 female). Statistical analyses testing the difference between bellbird and song thrush preen wax are inappropriate until among-species alignments are conducted (discussed in Chapter 4).

## **5.5 Discussion**

The results suggest preen wax from a conspecific or a bellbird does not affect song thrush incubation behaviour within 3 or 24 h after application to the nest lining and, therefore, do not support the hypothesis that odour cues emitted from preen wax play a role in detecting conspecific or intraspecific brood parasites at the nest in the song thrush. Since song thrush eject the eggs of the common cuckoo and conspecifics from their nests, a strong reaction to nest odour manipulations was expected if song thrush used odour to assess if their nest has been parasitised (Hale and Briskie 2007; Whittaker et al. 2009).

The findings differ from other studies that observed a change in incubation behaviour in response to nest odour manipulations (Amo et al. 2008; Mennerat 2008; Whittaker et al. 2009). Dark-eyed juncos reduced the length of their first incubation bout when preen wax from conspecifics or heterospecifics was applied to the nest

(Whittaker et al. 2009). Blue tits hesitated to enter their nest boxes upon their first return following odour manipulations with different nesting materials (Mennerat 2008) or the scent of a predator (Amo et al. 2008). By contrast, I found no significant difference in the length of the first visit to the nest among control and treatment trials in song thrush in this study. In dark-eyed juncos and blue tits, it was hypothesised that the change in behaviour was only seen when the bird first returned to the nest because the subjects habituated quickly to new odours and because the odour was likely the strongest at the beginning of the trial (Mennerat 2008; Whittaker et al. 2009). Eastern bluebirds *Sialia sialis*, however, did not avoid laying their clutches in nest boxes laced with snake or mammal odours (Godard et al. 2007).

The difference between my findings and Whittaker et al. (2009) may be because of where preen wax was applied to the nest. In an attempt to prevent the preen wax from being visually detected by the nest owner, I applied raw preen wax to the nest lining under the eggs. Alternatively, Whittaker et al. (2009) applied preen wax, diluted in acetone, to the eggs and to the rim of the nest. Preen wax odour may have been more noticeable on the nest rim than in the bottom of the nest to an incubating bird and is the likely location for preen wax to transfer from the feathers of a brood parasite to the nest when laying an egg. Alternatively, applying the preen wax on the rim of the nest could have also altered the nest visually if the reflectance of the preen wax altered the reflectance of the nest, and thus it is possible that Whittaker et al. (2009) confounded visual and odour cues, which was less likely in my trials. Future research should focus on the effect of the location of odours on the nest on host response.

It is possible song thrush detected the preen wax that was applied to nest lining under the eggs through taste or vision, especially since many of the song thrush put

their heads in their nests when turning or examining their eggs (Whittaker et al. 2009). However, this possibility is unlikely, because the preen wax was applied to nest lining below the eggs. The eggs would likely have prevented song thrush seeing the preen wax. Also, the likelihood of a song thrush putting their bills in the exact location I applied a preen wax or water treatment seems low.

The amount of preen wax applied to the nest in this experiment was likely more than would be typically transferred to the nest from the feathers of brood parasites during a parasitism event and thus was likely detectable by incubating female song thrush. However, some of the compounds in song thrush and bellbird preen wax may have short retention times and thus are likely to affect nest odour for only a certain period of time. It is possible that there was no difference between the control and treatment trials because the subjects may have returned to the nest only after the volatile and odorous molecules in the preen wax sample had eluted completely. The elution time of volatile and odorous compounds in song thrush preen wax is not known. Compounds in dark-eyed junco preen wax have molecular weights that range from 120–320 Da and are eluted within the retention time range of 10–60 mins (Whittaker et al. 2009). However, these elution times were measured under analytical conditions, which are hotter than the conditions experienced in the nest lining under the eggs. The average time it took song thrush to return following the application of preen wax to their nests was  $8.1 \pm 2.4$  min with a maximum of 24.9 min. Measurements using Headspace<sup>TM</sup> technologies and GC-MS as well as behavioural experiments measuring the response of avian subjects over time after application of preen wax to the nest are needed to determine the length of time odorous molecules are emitted from a specific amount of preen wax and to better understand the detectability of preen wax odour by subjects over time.

Differences in odour among the eggs in a clutch may also be required for eliciting anti-parasitic behaviour in the song thrush just as visual differences are required among the eggs in a clutch are required to elicit ejection (Honza et al. 2007; Cassey et al. 2008; Poláčiková and Grim 2010). Many species parasitised by avian brood parasites recognise and reject parasitic eggs on the basis of differences between the learned appearance of their own eggs and the appearance of the parasite's egg (Sealy and Underwood 2012). My experiment changed the smell of the nest and not the odour of only a single egg and thus did not provide subjects with an egg that smelled different than the rest. It is possible that anti-parasitic activities, such as egg ejection, may be triggered only if there is one egg that smells different than the others.

In conclusion, the results suggest song thrush do not respond to conspecific or heterospecific (i.e., bellbird) preen wax on the nest with changes in their incubation behaviour within 3 or 24 h of returning to the nest. Future research elucidating the temporal and spatial effect of preen wax on avian body odour, in terms of odour of the bird itself and the odour it leaves behind after visiting a nest, is required to better understand how birds use body odour to assess risk at the nest. In addition, testing the effect of common cuckoo preen wax on song thrush incubation behaviour is warranted.

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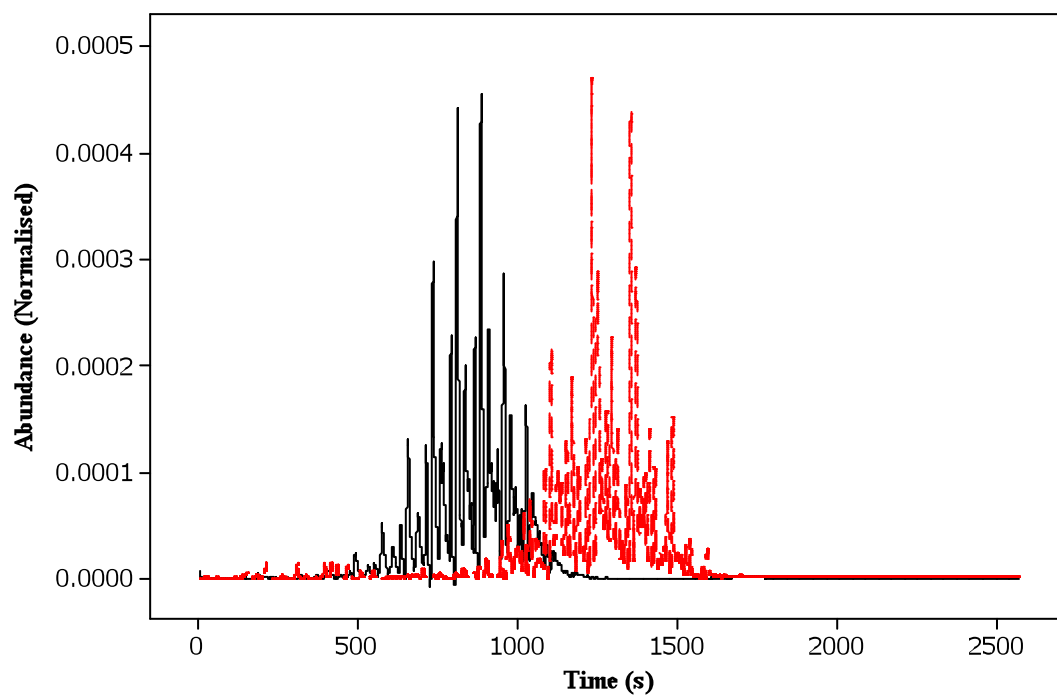
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Table 5.1. Incubation behaviour measurements of female song thrush in response to the addition of foreign song thrush or bellbird preen wax or a control treatment (i.e., water) to the bottom of the nest. Values are reported as means  $\pm$  SE. (PW = preen wax trial; C1 = control trial 1; C2 = control trial 2).

Variable	Group	n	Control trial 1	Preen wax trial	Control trial 2	Friedman ANOVA	
						$\chi^2$	p
Time present at nest (s)	Song thrush preen wax	12	7105.5 $\pm$ 267.5	7520.7 $\pm$ 226.2	7409.3 $\pm$ 295.2	0.50	0.78
	Bellbird preen wax	7	6328.6 $\pm$ 856.7	7613.7 $\pm$ 461.0	7480.9 $\pm$ 583.3	2.00	0.37
Peers per hour at nest	Song thrush preen wax	12	3.4 $\pm$ 0.6	3.5 $\pm$ 0.3	3.6 $\pm$ 0.5	0.50	0.78
	Bellbird preen wax	7	3.9 $\pm$ 1.7	3.3 $\pm$ 1.2	3.0 $\pm$ 0.6	1.56	0.46
Probes per hour at nest	Song thrush preen wax	12	4.6 $\pm$ 0.3	6.0 $\pm$ 0.6	6.3 $\pm$ 0.8	1.50	0.47
	Bellbird preen wax	7	3.3 $\pm$ 0.9	3.6 $\pm$ 0.8	5.7 $\pm$ 1.0	1.14	0.57
Percentage of time peering when at nest	Song thrush preen wax	12	0.22 $\pm$ 0.05	0.17 $\pm$ 0.03	0.18 $\pm$ 0.03	0.17	0.92
	Bellbird preen wax	7	0.22 $\pm$ 0.13	0.13 $\pm$ 0.05	0.16 $\pm$ 0.03	1.56	0.46
Percentage of time probing when at nest	Song thrush preen wax	12	0.89 $\pm$ 0.08	1.92 $\pm$ 0.68	1.62 $\pm$ 0.28	4.67	0.10
	Bellbird preen wax	7	0.48 $\pm$ 0.17	0.80 $\pm$ 0.27	1.02 $\pm$ 0.24	3.43	0.18
Length of first visit (s)	Song thrush preen wax	12	1617.0 $\pm$ 291.7	2021.5 $\pm$ 227.6	1889.3 $\pm$ 289.8	2.00	0.37
	Bellbird preen wax	7	1144.1 $\pm$ 483.3	2099.1 $\pm$ 558.5	1931 $\pm$ 409.3	2.00	0.37
Latency (s)	Song thrush preen wax	12	665.7 $\pm$ 139.4	463.2 $\pm$ 145.5	666.7 $\pm$ 303.8	0.17	0.920
	Bellbird preen wax	7	1300.7 $\pm$ 613.1	525.3 $\pm$ 136.9	742.7 $\pm$ 156.5	0.86	0.651

Figure 5.1. Mean gas chromatography (GC-FID) traces for preen wax from breeding adult bellbirds (black;  $n = 11$ , 6 females and 5 males) and song thrush (red;  $n = 3$ , 2 males and 1 female) collected in Kowhai and Waimangarara Bushes, South Island, New Zealand. Traces are not aligned among species (see Chapter 4).





## Chapter 6: General Discussion

Obligate avian brood parasitism is a rare reproductive strategy among birds which involves only about 1% of all c. 10,000 bird species (Rothstein and Robinson 1998; Davies 2000). Obligate avian brood parasites rely solely on other species to incubate their eggs and raise their young. Raising a brood parasite involves a cost to the host in terms of time, energy, and reproductive output (Øien et al. 1998; Rothstein and Robinson 1998; Davies 2000; Lorenzana and Sealy 2001). The reproductive cost incurred by hosts when raising a brood parasite sets off a evolutionary arms race, where hosts evolve adaptations to mitigate the cost of parasitism which in turn selects for improved adaptations in parasites to trick hosts into accepting their eggs and offspring (Dawkins and Krebs 1979; Rothstein and Robinson 1998; Davies 2000, 2011). Theoretically, this evolutionary arms race can continue into perpetuity or until a stable evolutionary end point is reached (Janzen 1980; Futuyma 1998).

This thesis investigated interactions between brood parasites and their hosts with the goal of improving our understanding of variations in host diversity among brood parasites and the evolutionary trajectory of the evolutionary arms race between brood parasites and their hosts. I took a two-pronged approach in this thesis. Chapter 2 and Appendix 1 specifically addressed questions about host specificity, using the shining cuckoo *Chrysococcyx lucidus lucidus* and the grey warbler *Gerygone igata* as a model parasite-host system, whereas Chapters 3, 4, and 5 addressed questions about coevolutionary interactions between hosts and parasites, using host-parasite systems in New Zealand and North America. In addition, in Appendix 2, I presented the

results of an experiment testing the response, in terms of changes in incubation behaviour, of red-winged blackbirds *Agelaius phoeniceus* to real and artificial brown-headed cowbird eggs.

Host diversity among brood parasites varies dramatically and the reasons are not well understood (Rothstein et al. 2002; Briskie 2003; Mermoz and Fernandez 2003; De Mársico and Reboreda 2008). Chapter 2 tested the hypothesis that aggressive nest defence among potential host species towards parasites may limit host diversity in some species of brood parasites. In other words, it tested the prediction that avian brood parasites may avoid the nests of species at which they are attacked aggressively. I predicted that species that are used as hosts would be less aggressive than potentially suitable host species that are not used as hosts if aggressive nest defence has an effect on host diversity in avian brood parasites.

I compared the nest defence response of grey warblers, the only host of the shining cuckoo in mainland New Zealand, to the nest defence response of South Island fantails *Rhipidura fuliginosa fuliginosa* (hereafter fantails) and silvereyes *Zosterops lateralis*, two potentially suitable host species but unused by the shining cuckoo, to the presence of a shining cuckoo and an innocuous species at the nest. The results suggested that grey warblers are as aggressive as fantails and silvereyes and thus do not support the prediction that nest defence behaviour limits host diversity among host species at least in New Zealand. However, the results suggest that shining cuckoos may have a better chance of accessing the nests of grey warblers and silvereyes without being detected than the nests of fantails because grey warblers and silvereyes took more time to return to their nests after I installed a model than fantails. In addition, the results suggest grey warblers and fantails recognise shining cuckoos

as different from an innocuous species (i.e., the chaffinch *Fringilla coelebs*) because they responded more aggressively towards shining cuckoo models.

The results presented in Chapter 2 represent the average level of aggression a shining cuckoo is likely to suffer when visiting the nest of a grey warbler, fantail, or silvereye. However, large variations in response were observed within each species, which makes generalisations about species-specific responses difficult (Mclean 1987). At some nests the models were attacked vigorously whereas at other nests the models were not attacked at all. The variations in response among nests suggests that nest defence may be a conditional response that is dependent on various factors such as the nest owners' previous experience (Campobello 2008; Davies and Welbergen 2009; Čapek et al. 2010; Langmore et al. 2012), age (Smith et al. 1984), or physical condition (Hogstad 1993, 2005). Additionally, the nest defence response may differ according to whether the male, female, or both sexes are present (Požgayová et al. 2009) and to the stage of the breeding cycle (Briskie and Sealy 1989). The sex of the first individual to return to the nest as well as the age, previous experience with shining cuckoos, and physical condition of the nest owners tested in Chapter 2 was not known. Testing the response of colour banded individuals of known age, sex and breeding history could increase our understanding of the interaction between shining cuckoos, grey warblers and other potentially suitable host species that are not currently used by shining cuckoos. Large variations in response between different individuals, however, is nearly universal in studies of nest defence, and the cause of most of this variation is unknown even after conditions such as age and sex are controlled for (Montgomerie and Weatherhead 1988).

This research can also be expanded by testing the nest defence behaviour of other suitable host species that are currently not used as hosts by shining cuckoos such

as the New Zealand robin *Petroica australis*, bellbird *Anthornis melanura*, brown creeper *Mohoua novaeseelandiae*, and tomtit *Petroica macrocephala*. These tests would reveal if nest defence behaviours of other species prevent shining cuckoos from accessing their nests. However, aggressiveness may not be the only factor that determines the effectiveness of nest defence behaviours. The effectiveness of nest defence behaviours may also be a function of the amount of injury a species can inflict on an intruder. In other words, species that are capable of inflicting serious injury with fewer strikes may not respond as aggressively but still be as effective at repelling brood parasites from their nests because the parasite has evolved to recognise these species as capable of inflicting injury with fewer strikes. Tests measuring the amount of damage grey warblers and potentially suitable host species can inflict on shining cuckoos are warranted. The ability of species to inflict damage on intruding shining cuckoos could be measured relatively by presenting shining cuckoo replicas made of Styrofoam at the nests of various species and recording the amount of damage that is inflicted upon them over pre-determined period of time.

In Appendix 1, I tested the hypothesis that host diversity of the shining cuckoo is limited by the availability of suitable alternative host species that do not possess anti-parasite adaptations. The results of two cross-fostering trials where shining cuckoos were inserted into fantail nests suggest that fantails do not eject shining cuckoo nestlings as has been reported in hosts of other *Chrysococcyx* spp. (Sato et al. 2010; Tokue and Ueda 2010). The results also suggest that fantails were as capable as grey warblers at raising a shining cuckoo nestling for at least 11 days in one cross-fostering trial and 15 days in the second trial (statistical analyses were inappropriate because of the small sample sizes). More cross-fostering experiments are necessary to properly assess the relative reproductive success of shining cuckoos

among available and potentially suitable host species in New Zealand. New Zealand is the ideal location to study if reproductive success among hosts limits avian brood parasites from parasitising more species or if some brood parasites specialise on one or a few hosts despite the potential for equal or greater reproductive success with other species, because it is relatively depauperate in terrestrial passerines (Briskie 2003).

Chapter 3 investigated the correlation between recognition and rejection of the eggs of parasites by hosts. Rejection of parasitism is composed of two parts: the recognition of parasitism and the response to parasitism such as nest desertion or ejection of parasite's eggs (Moskát and Hauber 2007). Previous studies have focused primarily on the response component of rejection because it shows that the host recognises parasitism because it responded (Moskát and Hauber 2007). Fewer studies have attempted to determine if some species recognise parasitism without responding with rejection (Moskát and Hauber 2007). Rejection by hosts that recognise parasitism may be context-dependent and based on such factors as the individual host's perceived risk of parasitism, the risk of recognition errors, and the cost of rejection (Øien et al. 1999; Avilés et al. 2005; Servedio and Hauber 2006; Moskát and Hauber 2007).

Studies investigating the incubation behaviour of yellow warblers *Setophaga petechia*, eastern olivaceous warblers *Iduna pallida*, and rufous-tailed scrub robins *Cercotrichas galactotes*, three species that reject the eggs of parasites at intermediate frequencies, suggest that some individuals recognise the eggs of parasites but did not reject them. Yellow warblers spent more time peering at and probing their clutches as well as more time shuffling while settling on the nest after the addition of artificial cowbird egg than before which suggests they recognise the eggs of brood parasites

(Guigueno and Sealy 2012). Likewise, eastern olivaceous warblers pecked clutches more often when parasitised with a common cuckoo *Cuculus canorus* egg (Antonov et al. 2009). Rufous-tailed scrub robins also appear to recognise the eggs of parasite because they pecked only foreign eggs that were experimentally added to their nest, did not peck their own eggs after the ejection of a foreign egg, and did not peck any eggs at control nests (Soler et al. 2012). However, in all three species, recognition did not always lead to rejection (Antonov et al. 2009; Guigueno and Sealy 2012; Soler et al. 2012).

To improve our understanding of the relationship between incubation behaviour and recognition of parasitic eggs, I compared the response, in terms of changes in incubation behaviour, to the presence of a brown-headed cowbird egg of a species that accepts the eggs of brown-headed cowbirds almost invariably (Rothstein 1975; Ortega and Cruz 1988; Rasmussen et al. 2010), the red-winged blackbird (Capper et al. 2012), to the response of a species that ejects the eggs of brown-headed cowbirds almost invariably, the gray catbird *Dumetella carolinensis* (Lorenzana and Sealy 2001). I hypothesised that if they recognise the brown-headed cowbird egg as a threat to their reproductive success, the changes in incubation behaviour in response to parasitism by red-winged blackbirds would be similar to the changes in incubation behaviour of gray catbirds, a species that obviously recognises the eggs of brown-headed cowbirds because they reject them at a very high frequency.

The results suggested that red-winged blackbirds do not recognise the eggs of brood parasites because their response was negligible in comparison to the response of gray catbirds. The findings of this study suggest the acceptance of brown headed cowbird eggs by red-winged blackbirds is not a conditional response because red-winged blackbirds did not appear to recognise them, at least within 3 hours after their

insertion in the nest. However, it is not known if the negligible response from red-winged blackbirds to the presence of an artificial brown-headed cowbird egg was because they did not recognise the egg and thus did not respond, or because they did not respond despite recognising the egg. Future research could investigate if the heart and respiratory rates of red-winged blackbirds change in response to the presence of a brown headed cowbird egg in the nest. Physiological changes associated with stress, such as changes in heart and respiratory rates, can be used to describe an individual's perception of external stimuli (Hugdahl 1995). Several studies have investigated the relationship between auditory stimuli and heart rate in birds (Searcy 1992). For example, heart rates have been used to describe the perception of conspecific calls in mallard ducklings *Anas platyrhynchos* (Evans and Gaioni 1990), the sex-specific perception of song in Bengalese finches *Lonchura striata* (Ikebuchi et al. 2003), the perception of the songs of conspecifics in European blackbirds *Turdus merula* (Diehl and Helb 1986), and the perception of allopatric and sympatric songs of conspecifics in chiffchaffs *Phylloscopus collybita* (Zimmer 1982). As such, indices of stress, such as changes in heart and respiratory rate, may reveal recognition of parasitism in red-winged blackbirds.

The results of the red-winged blackbird experiment and all experiments using only artificial cowbird eggs should be interpreted with caution because the response of birds to real and artificial cowbird eggs may be different. However, the availability of freshly laid parasitic eggs and the sample sizes required for scientific studies have necessitated the use of artificial eggs by researchers. In Appendix 2, I present the findings of a study comparing the responses of red-winged blackbirds, in terms of changes in incubation behaviour, to real and artificial eggs. Red-winged blackbirds responded, in terms of changes in their incubation behaviour, differently to real and

artificial cowbird eggs. However, the results do not change my interpretation of the findings in Chapter 3 because the findings do not suggest red-winged blackbirds perceived real brown-headed cowbird eggs as a threat because nest investigation behaviours (i.e., peering and probing) did not increase when a brown-headed cowbird egg was in the nest.

In Chapters 4 and 5, I present the results of two studies investigating the possible role of olfaction in avian brood parasite-host systems, which, to my knowledge are the first studies to do so. Apart from vultures, kiwis, honeyguides and seabirds, the use of olfaction by birds has historically been ignored by biologists but recent research has provided compelling evidence for extremely sophisticated olfactory abilities in many avian species in a variety of socio-ecological contexts (Clark et al. 1993; Steiger et al. 2008; Balthazart and Taziaux 2009; Steiger et al. 2009). Olfaction has been found to play a role in foraging (Goldsmith and Goldsmith 1982; Harriman and Berger 1986; Ioalé and Papi 1989; Nevitt et al. 1995; Nevitt 2000; Nevitt et al. 2008), predator avoidance (Hagelin et al. 2003; Amo et al. 2008; Roth et al. 2008 but see Godard et al. 2007), selection of nest materials (Clark and Mason 1985, 1987; Clark and Smeraski 1990; Malakoff 1999; Petit et al. 2002; Gwinner and Berger 2008; Mennerat 2008), homing (Wallraff 2003, 2004; Gagliardo et al. 2009; Gagliardo et al. 2011) as well as breeding colony and nest finding (Grubb 1973, 1974, 1979; Benvenuti et al. 1993; Minguez 1997; Bonadonna et al. 2001; Bonadonna and Bretagnolle 2002; Bonadonna et al. 2003; Bonadonna and Nevitt 2004; Caspers and Krause 2011). Birds also appear to gain important socio-ecological information on species, sex, and individual identity of other birds through olfaction (Cohen 1981; Hagelin et al. 2003; Jones et al. 2004; Hagelin 2007; Hagelin and Jones



2007; Whittaker et al. 2009; Zhang et al. 2009; Mardon et al. 2010; Zhang et al. 2010; Coffin et al. 2011; Whittaker et al. 2011).

The key source of avian body odour is believed to be preen wax because it is spread over the feathers during preening and because it contains volatile substances (Hagelin and Jones 2007; Soini et al. 2007; Campagna et al. 2012). The composition of preen wax is complex and has been found to vary among species, populations, and individuals, with age and reproductive status, as well as between the sexes. The variations in preen wax composition among species as well as the demonstrated ability of species to detect the difference in preen wax may act as a cue for discrimination between hosts and brood parasites.

Avian brood parasitism and the evolutionary arms race have produced some of the most convincing examples of mimicry in nature as seen, for example, in the remarkable similarity of the eggs of the majority of common cuckoo species and the eggs of their host (Brooke and Davies 1988; Davies and Brooke 1989; Davies 2000), the mouth markings of nestling parasitic finches and the mouth markings of the nestlings of their hosts (Payne 2005 but see Hauber and Kilner 2007), the appearance of nestling *Chalcites/Chrysococcyx* cuckoos and the appearance of the host nestlings, and of the begging calls of shining cuckoos and grey warbler nestlings (McLean and Waas 1987; Anderson et al. 2009). Mimicry in avian brood parasite systems is thought to have evolved in response to ever improving discrimination by hosts (McLean and Waas 1987; Davies 2000; Hauber and Kilner 2007; Anderson et al. 2009) or in response to nestling competition for parental provisioning (Hauber and Kilner 2007).

In Chapter 4, I compared the gas-chromatography flame ion detection (GC-FID) traces of the preen wax of the shining cuckoo, the grey warbler and six other

potentially suitable host species for evidence of mimicry between the shining cuckoo and the grey warbler. The comparisons suggested that the preen wax composition of the grey warbler is more similar to the preen wax composition of the shining cuckoo than to the preen wax composition of any of the other seven species examined which indicates that mimicry of the composition of grey warbler preen wax by shining cuckoos is likely. However, until I am able to analyse the data using a mass spectrometer to identify the compounds in the preen wax, this conclusion is tentative.

Selective pressures other than host discrimination such as environmental influences and even chance could have caused the similarity between the preen wax of shining cuckoos and grey warblers (Grim 2011). Experiments testing the response of grey warblers to odour-manipulated nestlings are necessary to determine mimicry. Odour manipulations using preen wax of the nestlings of species with dissimilar preen wax composition would be ideal because it would be an odour found in nature that is within the limits of nestling preen wax variability. To my knowledge, the study presented in Chapter 4 is the first study to investigate the use of olfaction in avian brood parasite-host systems directly by studying a potential source of odour in a brood parasite directly.

In Chapter 5, I presented the results of an experiment that tested the response of song thrush *Turdus philomelos* to nest-odour manipulations. Song thrush reject the eggs of the common cuckoo and intraspecific parasites at intermediate and low frequencies, respectively. I measured the effect of the preen wax of conspecifics and of a non-parasitic and non-predatory species, the bellbird, added to the nest on the incubation behaviour of female song thrush. If females use olfactory cues to detect parasitism, I expected that they would investigate their clutches more after the experimental addition of a foreign odour. I predicted that a change in the odour of

their nest, especially the odour of another bird, would alert the nest owner to the possible visit of another bird to their nest and based on this information they would investigate their clutch visually more frequently to determine if they had been parasitised. However, the results suggested that song thrush do not use odour to detect brood parasitism. The findings conflict with a previous study, to my knowledge the only other study to test the response of a passerine to the addition of preen wax to the nest, that found dark-eyed juncos *Junco hyemalis* responded to the addition of foreign preen wax to the nest (Whittaker et al. 2009). However, the response was only observed during the first incubation bout, which was shorter when foreign preen wax was added to the nest than during control trials. The short-term response of dark-eyed juncos was hypothesised to have occurred only when the bird first returned to the nest because that was when the odour would have been the strongest and because they may quickly habituate to the odour (Whittaker et al. 2009). By contrast, there was no significant difference in the length of the first visit of song thrush, which further suggests they did not detect a change in odour (Table 5.1). The different findings on the use of odour in dark-eyed juncos and song thrush validate the need for further experiments testing the use of olfaction in more species. The pattern of odour-use among hosts and among brood parasite systems may reveal the conditions required for the evolutionary arms race to include olfaction.

As with the lack of response to the addition of an artificial brown-headed cowbird egg to their nest by red-winged blackbirds in Chapter 2, it is not known if song thrush detected the odour but did not respond or if they simply did not detect the preen wax on the nest (Guigueno and Sealy 2012). Physiological changes such as changes in heart and respiratory rate of song thrush in response to changes in nest odour in song thrush may reveal recognition without the expected increase in

investigation of the nest contents. In addition, odour controlled experiments measuring the change in incubation behaviour in response to the addition of foreign eggs to their nests (i.e., a visual stimuli) would further determine the importance of preen wax odour to the detection of brood parasitism by song thrush. If song thrush respond to the addition foreign eggs to their nest we can conclude that visual stimuli are much more important to song thrush than olfactory stimuli for determining the risk of parasitism. Also, experiments testing the rejection or acceptance of foreign eggs over a 5-day acceptance period, the standard period of time to determine acceptance or rejection of parasitic eggs in the field of avian brood parasitism (Rothstein 1975), in response to nest odour manipulations may reveal that olfactory cues may be used in tandem with visual cues.

The addition of investigations on the use of olfaction in avian brood parasites systems can contribute to a fuller understanding of brood parasite-host coevolution. To date, all research has focused on only visual (including ultraviolet wavelengths)(Brooke and Davies 1988; Davies and Brooke 1989; Underwood and Sealy 2006; Honza et al. 2007; Cassey et al. 2008; Honza and Poláčiková 2008; Underwood and Sealy 2008; Langmore et al. 2011; Sealy and Underwood 2012), and auditory channels of communication (Davies et al. 1998; Hauber et al. 2001; Hauber et al. 2002; Ranjard et al. 2010; Gloag and Kacelnik 2013; Kleindorfer et al. 2013) and to a lesser extent on tactile channels (Marchetti, 2000), but has completely ignored another main channel, namely olfactory communication, despite the evidence of its importance in social parasitism in insects (Lenoir et al. 2001; Strohm et al. 2008; Bauer et al. 2010). Only by considering all channels of communication can we be assured we fully understand the full range of interactions between the two parties, and the adaptations and counter adaptations that have arisen as a result.

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## **Appendix 1: Are grey warblers *Gerygone igata* the optimal host of the shining cuckoo *Chrysococcyx lucidus* in New Zealand?**

### ***A1.1 Abstract***

Variations in host diversity among avian brood parasites may have evolved in response to variations in the ability of available host species to successfully raise young parasites. In this appendix, I present the findings of two cross-fostering trials. I cross-fostered a shining cuckoo egg into a fantail nest and I cross-fostered a shining cuckoo nestling into a fantail nest. The purpose of the trials was to assess the reproductive success of the shining cuckoo *Chrysococcyx lucidus lucidus*, a brood parasite that parasitises the nests of grey warblers *Gerygone igata* exclusively in mainland New Zealand, in the nests of a potentially suitable alternative host species, the South Island fantail *Rhipidura fuliginosa fuliginosa* (hereafter fantail). Cross-fostering trials revealed that fantails are capable of incubating the eggs and raising the nestlings of shining cuckoos. The growth rate of one of the cross-fostered shining cuckoo nestlings was very similar to the growth rate of shining cuckoo nestlings in grey warbler nests but the growth rate of the other cross-fostered shining cuckoo nestling was lower. More cross-fostering trials are required to assess the reproductive success of the shining cuckoo in fantail nests but my preliminary results suggest host specificity in shining cuckoos in New Zealand may not be limited by the lack of suitable alternative host species.

### ***A1.2 Introduction***

Why some species of brood parasite use a limited diversity of host species while others adopt a more generalist strategy is not well understood (Rothstein et al. 2002; Briskie 2003; Mermoz and Fernandez 2003; De Mársico and Reboreda 2008). Differential reproductive success among available host species has been suggested as a selective agent for specialisation in avian brood parasites (Rothstein et al. 2002; De Mársico and Reboreda 2008). In other words, brood parasites such as the shining cuckoo *Chrysococcyx lucidus lucidus* on the main islands of New Zealand might specialise on hosts such as the grey warbler *Gerygone igata* and avoid other species because it is with this host species that they obtain the highest reproductive success. Previous studies that have assessed the reproductive success of specialist parasites have done so in a small proportion of alternative host species (Kozlovic et al. 1996; Fraga 1998; Grim 2006; De Mársico and Reboreda 2008), in alternative host species that differed in body size from the primary host (De Mársico and Reboreda 2008), only during the egg stage with artificial cuckoo eggs (Briskie 2003), or only during the nestling stage of hosts that usually eject foreign eggs (Grim 2006). Therefore, a more thorough assessment of the reproductive success of host-specialist parasite species in alternative host species is warranted. New Zealand, being naturally depauperate in passerine species (the main group that act as hosts of parasitic cuckoos), is the ideal location for comparing the reproductive success of avian brood parasites in primary and alternative host species because it can more easily be assessed in a greater proportion of alternative host species than in other locations.

I tested whether host diversity is constrained by differences in reproductive success among host species (Fraga 1998; Rothstein et al. 2002) by comparing the

reproductive success of the shining cuckoo in nests of its primary host, the grey warbler, and in nests of an alternative host species that is not currently parasitised, the fantail *Rhipidura fuliginosa* (Gill 1998; Briskie 2003). I did this by measuring the success of two shining cuckoo nestlings in the nests of fantails. This potentially suitable but non-used host species is widely distributed throughout the shining cuckoos breeding range, breeds in the same habitats, has an insectivorous diet, and breeds at the same time as grey warblers (Heather and Roberston 2000).

### ***A1.3 Methods***

#### *Study site*

The study was conducted in Kowhai Bush (42°23' S, 173°37' E) and Waiman Bush (42°20' S, 173°40' E), New Zealand. Shining cuckoos are migratory and are common at these sites from October to January only whereas grey warblers and fantails are not migratory and are common at these sites year-round (Gill 1998; Briskie 2003; Higgins et al. 2006).

#### *Cross-fostering protocol*

Cross-fostering experiments were conducted to assess the reproductive success of the shining cuckoo in nests of a potentially suitable alternative host species. I cross-fostered a freshly-laid shining cuckoo egg from a grey warbler nest to a fantail nest. I also cross-fostered a shining cuckoo nestling on the day it hatched to a fantail nest on the day the eggs were hatching. In addition, I cross-fostered a shining cuckoo egg that had been incubated for an unknown period of time but was not going to hatch because



the grey warbler chicks hatched between four and five days before I removed the egg from the grey warbler nest. I attempted to make use of this egg by inserting it in the only available fantail nest where four eggs were being incubated but the number of days of incubation was not known because the nest was found after the clutch was completed. The shining cuckoo ended up hatching four days after the first fantail hatched. The shining cuckoo nestling died within one day of hatching. I quantified nestling growth rates using body mass.

#### ***A1.4 Results***

*Success of a shining cuckoo egg cross-fostered into a fantail nest during the laying stage on the day it was laid in the grey warbler nest.*

On 8 Nov 2010 at 5:30PM NZST, I added a shining cuckoo egg to an active fantail nest that contained one egg. The shining cuckoo egg was laid on 7 or 8 Nov in a grey warbler nest in the laying stage. The shining cuckoo egg weighed 1.69 g. I did not remove a fantail egg when I inserted the shining cuckoo egg. However, shining cuckoos usually remove one host egg when laying their own. At clutch completion on 10 Nov, the fantail nest contained three fantail eggs and one shining cuckoo egg. As fantails often lay four eggs, the addition of the cuckoo egg did not increase clutch size beyond the incubation ability of the host. On 24 Nov between 7:30AM and 3:07PM one of the fantail eggs hatched and between 3:07PM and 8:15PM a second fantail egg hatched. Between 8:15PM on 24 Nov and 5:45AM on 25 Nov the shining cuckoo egg hatched. It took between 14 and 15 days for the shining cuckoo egg to hatch from the onset of incubation or 17 days from the day it was inserted in the nest. Fantails

usually commence incubation with clutch completion (Powlesland 1982). The third and last fantail hatched between 5:45AM and 11:55AM on 25 Nov. Between 10:10AM on 27 Nov and 7:30AM on 28 Nov two of the fantail nestlings went missing. Between 7:30AM and 11:40AM on 28 Nov the third fantail nestling was evicted from the nest by the shining cuckoo nestling. It is assumed that the shining cuckoo nestling evicted all three fantail nestlings. It took this shining cuckoo nestling 3-4 days to evict all of the fantail nestlings. On 9 Dec when the nestling was 15 days of age and weighed 15.3 g, it perched on the side of the nest. On 10 Dec at 9:05AM there was no sign of the nestling or the foster parents at the nest. It is not known if the shining cuckoo fledged or if it was depredated. No body or signs of depredation were found in or near the nest. This nestling did not grow at the same rate as other shining cuckoo nestlings in grey warbler nests (Fig. A1.1; Table A1.1).

*Success of a shining cuckoo nestling cross-fostered on the day it hatched into a fantail nest on the day the fantail eggs were hatching.*

On 5 Nov 2012 at 11:53AM NZST, I found a grey warbler nest with two eggs. I could not tell the identity of the eggs because I did not have my mirror with me. On 6 Nov 2012, the nest contained one grey warbler egg and one shining cuckoo egg. The shining cuckoo egg hatched between 9:42AM on 13 Nov and 9:07AM on 14 Nov. At 3:23PM on 14 Nov, I removed the shining cuckoo from the grey warbler nest. At 3:54PM, I added the shining cuckoo nestling to a fantail nest that contained three fantail nestlings. The fantail nest originally contained four fantail nestlings but I removed one, which was selected at random, and inserted it into the grey warbler nest from which the cuckoo was removed for another experiment. Three of these fantail nestlings hatched between 9:02AM on 13 Nov and 9:56AM on 14 Nov. The fourth

nestling hatched between 9:56AM and 2:52PM on 14 Nov. By 8:01AM on 16 Nov, one of the fantail nestlings was missing. I could not find its body below the nest. At 2:41PM on 16 Nov, a second fantail nestling was missing from the nest. It was recovered alive below the nest and euthanised. It weighed 2.21 g. At 6:49AM on 17 Nov, the shining cuckoo nestling was observed trying to evict the last remaining fantail nestling. By 2:56PM on 17 Nov, the last remaining fantail was found below the nest and was euthanized. It weighed 3.48 g. At 3:24PM on 25 Nov, fantails were seen feeding the shining cuckoo. At 7:30AM on 26 Nov, the shining cuckoo nestling was very lethargic and cold and the adult fantails were not around. I warmed the nestling with my hands and returned it to the grey warbler nest from which it was removed. It appears fantails abandoned this nestling between 11 and 12 days after it was introduced into their nest. The reason for the desertion is not known. This shining cuckoo's mass increased at the same rate as shining cuckoo nestlings in grey warbler nests (Fig. A1.1; Table A1.1).

#### *Shining cuckoo nestlings in grey warbler nests*

I measured the mass of six shining cuckoo nestlings in grey warbler nests (Fig. A1.1; Table A1.1). The survival of nestlings varied among nests with only two out of six likely fledging successfully. The other four nestlings were depredated between four and six days of age, six and seven days of age, ten and eleven days of age, and eleven and twelve days of age, respectively. The timing of eviction of the nest contents is known for only five of the nests. It took a shining cuckoo nestling between two and three days to evict all nest contents at two nests (three nestlings were evicted at one nest and two nestlings and one egg were evicted at the other nest), between two and five days at a nest where two nestlings were evicted, between three and four days

where two nestlings and one egg were evicted, and between four and five days where one nestling was evicted.

### ***A1.5 Discussion***

The growth curve of one of the cross-fostered shining cuckoos, the one that was incubated in a grey warbler nest and only transferred to a fantail nest on the day it hatched, was very similar to the growth curve of shining cuckoos in grey warbler nests (Table A1.1; Table A1.1). The growth curve of the other cross-fostered shining cuckoo nestling, the one that was incubated in a fantail nest, was lower, thus it always had a lower mass than the other cross-fostered shining cuckoo nestling and the shining cuckoo nestlings raised in grey warbler nests (Fig. A1.1; Table A1.1). Since only two proper cross-fostering experiments were conducted, it is difficult to make any conclusions about the suitability of fantails as hosts of the shining cuckoo. However, I can conclude that fantails do not appear to reject shining cuckoo nestlings immediately after they hatch and will likely provide them with parental care for several days after hatching. Ejection of parasitic nestlings soon after they hatch has been observed in large-billed gerygones *Gerygone magnirostris* and mangrove gerygones *Gerygone laevigaster* which both eject little-bronze cuckoo nestlings *Chrysococcyx minutillus* from their nests (Sato et al. 2010; Tokue and Ueda 2010). Likewise, superb fairy-wrens *Malurus cyaneus* have been reported to desert nestling Horsfield's bronze cuckoo *Chrysococcyx basalis* at 11 of 29 nests (11 of 42 when depredated nests are included) when the parasitic nestlings were between 3 and 6 days of age. Early rejection of cuckoo nestlings is likely to have been selected for to rid the

nest of the cuckoo nestling before it evicts the host eggs and nestlings. Fantails appeared to have deserted a shining cuckoo nestling when it was between 11-12 days of age, which is about same amount of time it takes to fledge a brood of fantail nestlings (12.5 days,  $n = 11$ , Powlesland 1982). It is possible that some fantail pairs abandon broods that are in the nest for a longer period of time than is typical for their own young which is a recognition-free and likely cost-free mechanism of rejection of parasitic young that frees them from the costs of raising a fledgling shining cuckoo (Anderson and Hauber 2007). However, further research is required to assess the presence of anti-parasite defences, such as this one, in fantails.

The results show that fantails are capable of incubating the eggs of shining cuckoos. The incubation period of a shining cuckoo egg in a fantail nest reported here is within the range of previously reported for shining cuckoo eggs in grey warbler nests. Gill (1983) reported the incubation period from the day the egg appeared in the nest (day 1) to the day it hatched, and this period ranged from 13 to 17 days. By following Gill's (1983) method of calculating the incubation period, the incubation period of the shining cuckoo inserted in the nest of a fantail was 17 days. This method, however, depends on when the egg was laid relative to the host's nest cycle. For example, if I had inserted the shining cuckoo egg later in the laying stage, such as the day the clutch was completed, the incubation period would only have been between 14 and 15 days, according to Gill's (1983) method.

The reported incubation period of shining cuckoo eggs (15.5 days,  $n = 3$ , Gill 1980) is shorter than the incubation period of grey warbler eggs (19.5 days,  $n = 14$ , Higgins and Peter 2002) but similar in length as the incubation period of fantail eggs (14.2 days,  $n = 32$ , Powlesland 1982). This means that the window of opportunity, in terms of parasitising the nest at the right time to ensure proper incubation, is much

shorter in the fantail than it is in the grey warbler. Since fantails and shining cuckoos have very similar incubation periods, shining cuckoos need to parasitise fantail nests during the laying stage in order for the eggs to hatch at the same time as the eggs of the fantail. Shining cuckoo eggs laid during the incubation stage of fantail nests may still hatch, but the fantail eggs will likely hatch before the shining cuckoo and the older and larger fantail nestlings will likely outcompete the shining cuckoo and possibly smother it as was seen in the trial where a shining cuckoo egg was inserted in the fantail nest during the incubation stage (see Methods). The results suggest that to successfully parasitise fantails, shining cuckoos must parasitise them during the laying stage, which is only three or four days depending on the size of the clutch. By comparison, the window of opportunity for successful parasitism of grey warblers is 7.5 to 9.5 days assuming an incubation period of 15.5 days for shining cuckoos and an incubation period of 19 days for grey warblers and laying periods of four to six days for clutches of three to four eggs, respectively (Gill 1980; Higgins and Peter 2002). However, McLean and Rhodes (1991) reported that two out of three shining cuckoo eggs laid about a week after incubation began hatched and were raised successfully which suggests the window of opportunity for successfully parasitising grey warblers may be a few days longer than I suggest above. Grey warbler lay their eggs at 48 h intervals whereas fantail lay their eggs at 24 h intervals (Gill 1980; Powlesland 1982). As a result, the probability of synchronising parasitism with host-laying to maximise hatching and nestling survival is much lower in fantails than it is in grey warblers.

Although fantails were capable of hatching a shining cuckoo egg, the difference in the growth rate between the shining cuckoo nestling that was incubated in a fantail nest and shining cuckoo nestling that was incubated in a grey warbler nest but raised by fantails suggests that the suitability of fantails as incubators of shining

cuckoo eggs requires further research. The incubation patterns of grey warblers and fantails differ dramatically as incubation in grey warblers is performed solely by females whereas it is performed by both males and females in fantails. Female grey warblers have been reported to spend on average 68% of their time incubating (range: 60-72%; Gill 1980) whereas male and female fantails share incubation almost equally and spend on average ~ 90% of their time on the nest during the incubation period, however, they spent only ~ 70% of their time on the nest during the first two days of the incubation period (Ude Shankar 1977). Differences in incubation behaviour between these two species are likely to result in differences in variations in temperature to which the eggs, and thus the embryos are exposed. Little is known about how temperature and temperature variations affect the development of embryos or its effects on the future survival (DuRant et al. 2013). However, studies on megapodes and waterfowl suggest incubation temperature and variations in incubation temperature have significant effect on phenotypic traits in hatchlings (DuRant et al. 2013). As a result, shining cuckoo embryos may require a specific thermal environment to maximise their future survival that is only available with some host species. Future research investigating the effect of incubation temperature on shining cuckoo on future growth and survival are warranted as are measurements of incubation temperature and variations in temperature among potential host species. The possible differences between the thermal environments between fantail and grey warbler nests during incubation may have been increased because I did not remove one fantail egg when I inserted the shining cuckoo egg. According to the incubation limit hypothesis (Davies and Brooke 1988; McMaster and Sealy 1997), cuckoos remove eggs to reduce the clutch to a size that can be incubated effectively by the host species.

The energy expenditure required to evict the hosts' eggs and nestlings may reduce the reproductive success of shining cuckoos in fantail nests (Anderson et al. 2009; Hargitai et al. 2012). The time to eviction of all of the hosts' eggs or nestlings by shining cuckoo nestlings was similar in both grey warbler and fantail nests and thus does not appear to prevent shining cuckoos from parasitising fantails. Shining cuckoo nestlings evicted all nest contents from grey warbler nests within two to five days ( $n = 5$ ; range = 2–5 days) whereas shining cuckoos evicted all nests contents from fantail nests in 3.5 days ( $n = 2$ ; range = 3–4 days). However, the mean eviction time of all nest contents by shining cuckoos in fantail nests may actually be a little bit longer because I removed one fantail nestling from one of the nests where I inserted a shining cuckoo nestling.

More cross-fostering trials are required to better assess the reproductive success of the shining cuckoo in fantail nests and nests of other abundant and potentially suitable host species that are not currently used as hosts such as the bellbird *Anthornis melanura*, brown creeper *Mohoua novaeseelandiae*, and New Zealand robin *Petroica australis*. New Zealand is the ideal location to study if reproductive success among hosts limits avian brood parasites from parasitising more species or if some brood parasites specialise on one or a few hosts despite the potential for equal or greater reproductive success with other species, because it is relatively depauperate in terrestrial passerines (Briskie 2003). These studies may reveal the presence of anti-parasitic adaptations, such as nestling rejection, in non-used hosts which may improve our understanding of past host-use patterns of the shining cuckoo or other brood parasites such as the long-tailed cuckoo *Eudynamys taitensis*.



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Figure A1.1. Mass (mean  $\pm$  SE) of shining cuckoos raised in grey warbler nests and in fantail nests. Sample sizes and values are presented in Table A1.1. Data points without error bars involve only one individual. The shining cuckoo nestling that was cross-fostered as an egg and incubated in a fantail nest is represented by the red line (A). The shining cuckoo that was cross-fostered to a fantail nest the day it hatched in a grey warbler nest is represented by the green line (B). Sample sizes in Table A1.1.

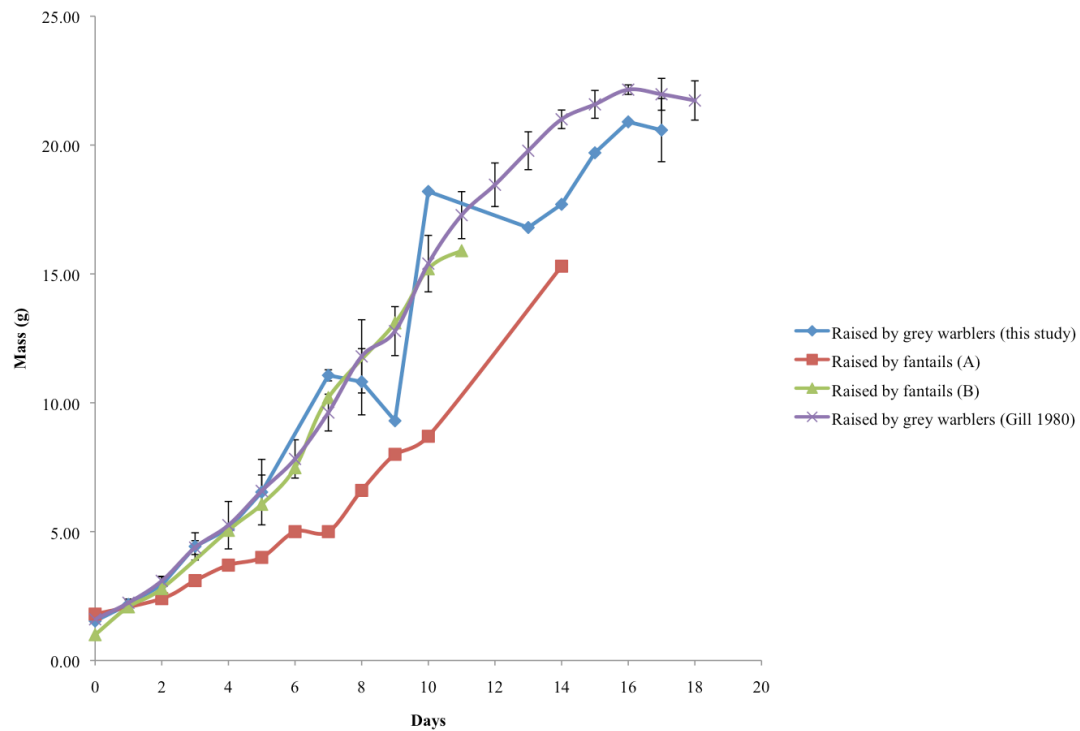


Table A1.1. Mass (mean  $\pm$  SE) of shining cuckoos raised in grey warbler nests and in fantail nests. Nestling A is a shining cuckoo nestling that was cross-fostered as an egg and incubated in a fantail nest. Nestling B is a shining cuckoo that was cross-fostered to a fantail nest the day it hatched in a grey warbler nest.

Age (Days)	Shining cuckoos raised by fantails		Shining cuckoos raised by grey warblers			Shining cuckoos raised by grey warblers		
	Nestling A	Nestling B	(This study)			(Gill 1980)		
	Mass (g)	Mass (g)	n	Mass (g)	SE	n	Mass (g)	SE
0	1.80	1.00	1	1.53		7	1.60	0.08
1		2.08	4	2.18	0.21	7	2.24	0.11
2	2.40	2.79	4	2.92	0.34	7	3.09	0.16
3	3.10		3	4.43	0.53	6	4.38	0.27
4	3.70	5.05	1	5.10		4	5.25	0.92
5	4.00	6.06	3	6.53	1.27	6	6.58	0.62
6	5.00	7.48				6	7.82	0.74
7	5.00	10.20	2	11.07	0.22	6	9.62	0.71
8	6.60		2	10.82	1.29	3	11.80	1.42
9	8.00	13.10	1	9.30		4	12.78	0.95
10	8.70	15.20	1	18.20		5	15.40	1.10
11		15.90				5	17.28	0.91
12						5	18.46	0.85
13			1	16.80		4	19.78	0.74
14	15.30		1	17.70		4	21.00	0.36
15			1	19.70		4	21.58	0.54
16			1	20.90		4	22.15	0.19
17			2	20.58	1.23	3	21.97	0.62
18						3	21.73	0.76

## **Appendix 2: Do hosts of avian brood parasites respond to artificial eggs and real eggs the same way in artificial parasitism experiments?**

### ***A2.1 Abstract***

The rarity of the eggs of avian brood parasites, in terms of the number required for scientific experiments, has necessitated the use of artificial eggs in research. Differences between artificial eggs and real eggs are inevitable and thus, controlled experiments comparing the response of hosts to artificial eggs and real eggs should be conducted to ensure the responses of hosts to artificial eggs are also those expected in response to real eggs. In this appendix, I compare the responses of red-winged blackbirds *Agelaius phoeniceus*, in terms of changes in incubation behaviour, to the presence of real and artificial brown-headed cowbird *Molothrus ater* eggs in their nests. The results suggest cowbird egg type (i.e., real or artificial) and changes in clutch size affect red-winged blackbird incubation behaviour, but not in a way that would be expected if they recognised the cowbird egg as a threat.

## ***A2.2 Introduction***

The rarity of the eggs of avian brood parasites, especially among the cuckoos, has necessitated the use of artificial eggs in scientific research (Rothstein 1970; Davies and Brooke 1989; Briskie 2003). Researchers have used wood (Alvarez et al. 1976; Wiley 1982; Davis et al. 2002), plaster-of-Paris (Rothstein 1975), plaster-of-Paris and glue (Soler et al. 2012), floral foam covered with a thin layer of plaster-of-Paris (Lee et al. 2005; Capper et al. 2012; Guigueno and Sealy 2012), wood-putty (Cruz and Wiley 1989), clay (Soler et al. 1995; Briskie 2003), and plastic (Higuchi 1989, 1998; Moskát and Fuisz 1999; Langmore et al. 2003) to make models which they then painted to give them the appearance of the eggs of parasites or to make them appear non-mimetic. Other researchers have even used the eggs of other species which they sometimes paint (Soler et al. 2012).

How well these artificial eggs mimic real eggs, especially in their ability to elicit the same responses from hosts, has received little attention. Appearance, shape, and size are likely the most important cues used by hosts for egg discrimination (Rothstein 1975; Underwood and Sealy 2006b) which can be mimicked fairly closely by artificial eggs (Prather et al. 2007), however, artificial eggs typically have rougher surfaces (Rothstein 1975) and the reflectance of ultra violet wavelengths by artificial eggs, which can be seen by birds but not by humans, is rarely considered (Cherry and Bennett 2001; Avilés et al. 2006; Cassey et al. 2008; Underwood and Sealy 2008; Avilés et al. 2010). Additionally, some of the materials used to make artificial eggs are hard and may physically preclude the actions some hosts use when faced with a real egg, such as puncture-ejection (Rohwer and Spaw 1988; Rohwer et al. 1989; Sealy and Neudorf 1995; Peer et al. 2005; Soler et al. 2012), and may greatly increase

the cost of rejection (Martín-Vivaldi et al. 2002). Therefore, the use of artificial eggs to assess the responses of hosts to brood parasitism may be inaccurate (Rothstein 1977; Underwood and Sealy 2006a).

Previous research that has compared the response of hosts to real and artificial eggs are not common (Prather et al. 2007). Yet, it is important to test the response of hosts to real and artificial eggs in every study to ensure that the artificial eggs being used elicit the same responses from the host under investigation as real eggs would, because model eggs are likely to vary in shape, size, appearance, texture, and mass among studies (Hale and Briskie 2007).

To control for the use of artificial eggs in Chapter 3, I tested the response of red-winged blackbirds *Agelaius phoeniceus*, in terms of changes in incubation behaviour, to the insertion of real and artificial brown-headed cowbird *Molothrus ater* (*hereafter* cowbird) eggs to their nests. I hypothesised that the differences in incubation behaviour of red-winged blackbirds among trials would be the same at nests tested with real cowbird eggs and at nests tested with artificial cowbird eggs.

### ***A2.3 Methods***

This study was conducted near the town of Delta, Manitoba in the marshes, farmlands, and forested dune-ridge on the south shore of Lake Manitoba, Canada (50°11'N, 98°19'W)(Mackenzie 1982). Red-winged blackbirds are abundant on the site during the spring and summer and are parasitised by cowbirds at a rate that varies from from 2–35% per year (Weatherhead 1989; Neudorf and Sealy 1994; Grant and Sealy 2002; Woolfenden et al. 2004; Capper et al. 2012).

The experimental protocol followed was exactly the same as the one described in Chapter 3. However, I describe it again here, almost in verbatim, for the sake of convenience. I tested the response of red-winged blackbirds, in terms of incubation behaviour, to the insertion of a cowbird egg in their clutch. A within subject-design was used to control for individual differences in incubation behaviour (Gravetter and Forzano 2003). Each nest was video recorded on 3 subsequent days. I waited 24 h between recordings to minimise carry over effects. On the first and third days, I recorded incubation behaviour at the nest without manipulating the clutch. These recordings served as control treatments (PRE and POST trials) for the experiment. On the second day, I recorded incubation behaviour immediately after I experimentally parasitised the nest by adding an artificial egg that was painted to appear like a cowbird egg to the clutch (CBE trial). To control for the change in clutch size and volume caused by the addition of a cowbird egg, I removed one host egg from nests for the duration of the CBE trial at 8 of the 23 red-winged blackbird nests where I used artificial eggs and 5 of the 10 red-winged blackbird nests where I inserted a real cowbird egg.

Video cameras were set on tripods ~ 5 m from the nest. Cameras were covered with burlap cloth in an attempt to help them blend in with the surrounding vegetation. It is not known how the subjects perceived the camera apparatus. However, any effect the camera apparatus may have had on the subjects was controlled by using camera apparatuses with similar appearances at each nest and for each trial. Sony® DCR cameras with extended life batteries and internal hard drives for data storage were used to video record nests. I quantified the behavioural data from all of the video recordings to eliminate inter-observer variability.



Real cowbird eggs were obtained from red-winged blackbird nests not used in the study except for one egg that was laid naturally in a nest on the day I was supposed to parasitise it with an artificial cowbird egg and video record it. All real cowbird eggs used in this study showed no signs of incubation. Artificial cowbird eggs are described in detail in the 'Methods' section of Chapter 3. Artificial eggs ( $n = 23$ ) and real eggs ( $n = 5$ ) used in red-winged blackbird nests did not differ significantly in either mass ( $3.22 \pm 0.07$  g vs.  $3.16 \pm 0.09$  g, Welch  $t = 0.53$ , d.f. = 9,  $p = 0.61$ ) but differed significantly in width ( $17.54 \pm 0.03$  mm vs.  $16.84 \pm 0.17$  mm,  $t = 4.0550$ , d.f. = 4,  $p = 0.02$ ), and length ( $22.93 \pm 0.08$  mm vs.  $20.62 \pm 0.29$ ,  $t = 7.6787$ , d.f. = 4,  $p = < 0.01$ ; Table A2.1).

In total, I video recorded 45 red-winged blackbird nests, but I used the data from only 33 of these. The video recordings taken at 12 of the 45 nests were excluded because the nests were obscured by vegetation (5 of 12 nests), or they were taken at nests at which I was unable to record incubation behaviour in response to all three trials (i.e., PRE, CBE, and POST trials) because of depredation (3 of 12 nests), human disturbance (2 of 12 nests), desertion (1 of 12 nests) or rain (1 of 12 nests). I tested 23 of the 45 nests with artificial eggs. The remaining 10 nests were tested with real cowbird eggs.

I video recorded incubation behaviour at red-winged blackbird nests in which I inserted artificial cowbird eggs from 21 May to 18 June 2012 and real cowbird eggs from 25 May to 15 June 2012. In total, I analysed 203.02 h of incubation behaviour at 23 nests where artificial cowbird eggs were used and 86.91 h at 10 nests where real cowbird eggs were used. For each trial I analysed an average of  $2.94 \pm 0.02$  h ( $n = 69$ , range: 1.83–3.00) of video for nests tested with artificial cowbird eggs and an average of  $2.89 \pm 0.06$  h ( $n = 30$ , range: 1.60–3.00) for nests tested with real cowbird eggs. All

variables are presented as percentages or rates to account for observation time differences among trials. At nests tested with artificial cowbird eggs, trials began as early as 5.23 min before sunrise and as late as 120.40 min after sunrise (mean =  $41.65 \pm 3.33$  min,  $n = 69$ ) but the maximum difference among trial start times at individual nests averaged  $24.80 \pm 4.23$  min ( $n = 23$ , range: 2.72–81.32). At nests tested with real cowbird eggs, trials began as early as 0.26 min and as late as 139.28 min after sunrise (mean =  $45.02 \pm 7.10$  min,  $n = 30$ ) but the maximum difference among trial start times at individual nests averaged  $22.08 \pm 5.40$  min ( $n = 10$ , range: 2.78–59.00). I tried to start trials as early as possible to sunrise to mimic natural parasitism by brown-headed cowbirds which lay shortly before sunrise (McMaster et al. 2004).

## ***A2.4 Results***

### *Real vs. artificial eggs*

Red-winged blackbirds responded to real and artificial cowbird eggs differently. Red-winged blackbirds tested with real cowbird eggs spent a significantly lower percentage of their time at the nest during the POST trial than during the PRE and CBE trials (Table A2.2; Wilcoxon matched-pairs test; PRE vs. POST:  $Z = 2.50$ ,  $p = 0.01$ ; CBE vs. POST:  $Z = 2.50$ ,  $p = 0.01$ ; PRE vs. CBE:  $Z = 1.27$ ,  $p = 0.20$ ). However, the percentage of time red-winged blackbirds spent at the nest did not differ significantly among trials when artificial cowbird eggs were used.

The percentage of time red-winged blackbirds spent probing differed significantly among trials at nests tested with real cowbird eggs but not at nests tested with artificial cowbird eggs (Table A2.2). However, post hoc tests revealed that the

only significant difference between trials was between the PRE and the POST trials ( $Z = 2.50$ ,  $p = 0.01$ ), but the differences between PRE and CBE ( $Z = 1.89$ ,  $p = 0.06$ ) and CBE and POST ( $Z = 1.89$ ,  $p = 0.06$ ) approached significance suggesting that the percentage of time red-winged blackbirds probed when parasitised with a real cowbird egg increased sequentially from the PRE to the POST trials.

There were significant differences among trials in probing frequency when red-winged blackbirds were tested with artificial eggs but not when tested with real eggs (Table A2.2). However, post-hoc tests revealed that the only significant difference in probing frequency between trials at nests tested with artificial eggs was between the CBE and the POST trial ( $Z = 2.89$ ,  $p = < 0.01$ ). No significant difference was found between the PRE and CBE trials ( $Z = 0.82$ ,  $p = 0.41$ ) or between the PRE and POST trials ( $Z = 1.22$ ,  $p = 0.22$ ).

*'Add' vs. 'Switch' manipulation type*

There were no significant differences in incubation behaviour among trials for either manipulation type (i.e., 'add' or 'switch') at nests tested with artificial cowbird eggs (Table A2.3). However, there were significant differences among trials at nests tested with real cowbird eggs where clutch size was not controlled (i.e., 'add') but there were no differences among trials at nests tested with real cowbird eggs where clutch size was controlled (i.e., 'switch'; Table A2.4).

The percentage of time red-winged blackbirds spent at the nest differed significantly among trials at nests tested with real cowbird eggs only at nests where clutch size was not controlled (i.e., 'add' manipulation) but not at nests where clutch size was controlled (i.e., 'switch' manipulation; Table A2.4). Post hoc tests suggest the percentage of time spent at the nest was significantly lower during the POST trial

than during the CBE trial ( $Z = 2.02$ ,  $p = 0.04$ ) or the PRE trial ( $Z = 2.02$ ,  $p = 0.04$ ) and, the percentage of time red-winged blackbirds spent at the nest did not differ significantly between the PRE and CBE trial when real eggs were used ( $Z = 1.21$ ,  $p = 0.23$ ). The percentage of time spent probing when at the nest differed significantly among trials at nests tested with real cowbird eggs only for those nests where clutch size was not controlled (i.e., 'add' manipulation) but not at nests where clutch size was controlled (i.e., 'switch' manipulation; Table A2.4). Post hoc tests suggest the percentage of time red-winged blackbirds spent probing when at the nest was significantly higher during the POST trial than during the PRE trial ( $Z = 2.02$ ,  $p = 0.04$ ) and the CBE trial ( $Z = 2.022$ ,  $p = 0.04$ ) and, the percentage of time red-winged blackbirds spent probing did not differ significantly between the PRE and CBE trial when real eggs were used ( $Z = 0.67$ ,  $p = 0.50$ ).

## ***A2.5 Discussion***

Red-winged blackbirds appear to perceive real and artificial eggs cowbird eggs differently. The percentage of time red-winged blackbirds spent at the nest decreased significantly and the percentage of time they spent probing when at the nest increased significantly after the removal of a real cowbird egg (i.e., between the PRE and POST observation periods) but not after the removal of an artificial egg. Probing frequency also decreased after the removal of artificial cowbird eggs but not after the removal of real cowbird eggs.

Manipulation type also appeared to have an effect on incubation behaviour, but only when real eggs were used. Significant decreases in the percentage of time

spent at the nest and percentage of time spent probing were recorded only at nests where real eggs were ‘added’ to the clutch but not at nests where real cowbird eggs were ‘switched’ for host eggs during the CBE trial. By contrast, there was no difference in incubation behaviour among trials for either manipulation type at nests tested with artificial cowbird eggs.

Red-winged blackbirds only changed their incubation behaviour after a real cowbird egg was removed from their nests and where the ‘add’ manipulation type was used which, because of the manipulation type, made it appear that their clutch size decreased by 1 egg (e.g. CBE trial = 1 real cowbird egg + host clutch, POST trial = host clutch – 1 real cowbird egg). By contrast, red-winged blackbirds did not change their incubation behaviour after an artificial egg was removed from their nests where the ‘add’ manipulation type was used, which because of the manipulation type, also made it appear that their clutch size decreased by 1 egg. This suggests that red-winged blackbirds perceived the real eggs differently than they perceived the artificial eggs. Likewise, the lack of significant differences among trials at nests where the ‘switched’ manipulation was used for nests tested with real cowbird eggs suggests that red-winged blackbirds may be responding to the decrease in clutch size between the CBE trial and the POST trial when the ‘add’ manipulation type was used, but only when real cowbird eggs are used.

The responses of red-winged blackbirds to clutch manipulations are similar to those of clay-colored sparrows *Spizella pallida* (Hill and Sealy 1994). Hill and Sealy (1994) tested the response of clay-colored sparrows to the presence of an adult parasite at the nest, several clutch manipulations which included sparrow and cowbird eggs, and the addition of broken eggshells in the nest. They found that only clutch

reductions were found to elicit a response, which was desertion in this case (Hill and Sealy 1994).

Clutch reduction was also the primary factor for nest desertion by the great reed warbler *Acrocephalus arundinaceus* but only where the initial clutch size was 3 eggs but not where the initial clutch size was 5 eggs (Moskát et al. 2011). Clutch reductions were completed by the removal of two host eggs by the observer or by the replacement of two host eggs with model cuckoo eggs by the observer followed by the subsequent ejection of the cuckoo eggs by the host (Moskát et al. 2011). Previous parasitism at the nest did not have an effect on nest desertion (Moskát et al. 2011). Moskát et al. (2011) support Hill and Sealy (1994)'s hypothesis that the selective pressure for desertion is clutch reduction and not brood parasitism, at least in clay-colored sparrows and great reed warblers. Likewise, it is not likely the changes in behaviour of red-winged blackbirds in response clutch reduction was selected for by the pressures of brood parasitism, especially since the cost of acceptance after the initial parasitism event is low in red-winged blackbirds (Clotfelter 1997; Lorenzana and Sealy 1999) and because they did not respond, in terms of changes in incubation behaviour, like an ejector to the insertion of a cowbird egg in their clutch (i.e., gray catbirds; see Chapter 3).

Additional experiments testing the effect of the addition and the removal of cowbird eggs and host eggs on host behaviour are warranted. Yet, it is also possible that the differences found among trials are simply an artefact of the small sample sizes, which are the result of subdividing the main sample into smaller subcategories (e.g. egg type, manipulation type). Further tests are required to increase the power of the statistical tests to strengthen the validity of results. In terms of animal ethics, researchers should also be vigilant to the effect of removing experimental eggs at the

end of trials (e.g. removal of an experimental egg after the 5-day acceptance period (Rothstein 1975).

The results do not change my interpretation of the results in Chapter 3 because the pattern of response to artificial or real cowbird eggs among trials by red-winged blackbirds does not suggest that they recognise the egg, whether real or artificial as a threat to their reproductive success. Nest investigation behaviour (i.e., probing and peering) did not increase when a cowbird egg, whether real or artificial, was present in the nest like it did in catbirds, a species that ejects the cowbird eggs. However, the findings may potentially affect the interpretation of other studies that used only artificial eggs. Additional tests on the species used in these studies should be conducted with real eggs to ensure the results are not simply an artefact of the use of artificial eggs.

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Table A2.1. Mean  $\pm$  SE of real and artificial cowbird egg dimensions and masses used in the nests of red-winged blackbirds for this study.

Host species	n	Width (mm)	Length (mm)	Mass (g)
Real cowbird eggs	5	16.84 $\pm$ 0.17	20.62 $\pm$ 0.29	3.16 $\pm$ 0.09
Artificial cowbird eggs	23	17.54 $\pm$ 0.03	22.93 $\pm$ 0.08	3.22 $\pm$ 0.07

Table A2.2. Red-winged blackbird behaviour at the nest according to experimental egg type during the “pre-cowbird egg” (PRE), “cowbird egg” (CBE) and “post-cowbird egg” (POST) observation periods. Values are reported as means  $\pm$  SE.

Variable	Egg type	n	PRE	CBE	POST	Friedman's ANOVA	
						$\chi^2$	p
Percentage of time present at nest	Real	10	86.31 $\pm$ 1.47	85.20 $\pm$ 1.48	81.07 $\pm$ 1.94	10.40	<b>0.01</b>
	Artificial	23	81.80 $\pm$ 1.73	84.22 $\pm$ 1.69	83.95 $\pm$ 1.38	0.26	0.88
Peers/h present at nest	Real	10	4.40 $\pm$ 0.59	4.59 $\pm$ 0.46	5.33 $\pm$ 0.55	5.60	0.06
	Artificial	23	4.74 $\pm$ 0.40	4.76 $\pm$ 0.46	4.81 $\pm$ 0.37	1.13	0.57
Percentage of time peering when at nest	Real	10	0.22 $\pm$ 0.04	0.34 $\pm$ 0.05	0.40 $\pm$ 0.04	5.60	0.06
	Artificial	23	0.26 $\pm$ 0.03	0.32 $\pm$ 0.07	0.27 $\pm$ 0.03	1.65	0.44
Probes/h present at nest	Real	10	6.38 $\pm$ 0.77	7.53 $\pm$ 0.98	7.07 $\pm$ 0.96	3.80	0.15
	Artificial	23	7.44 $\pm$ 0.85	7.80 $\pm$ 0.59	6.55 $\pm$ 0.46	7.91	<b>0.02</b>
Percentage of time probing when at nest	Real	10	1.32 $\pm$ 0.23	1.71 $\pm$ 0.24	2.45 $\pm$ 0.41	8.60	<b>0.01</b>
	Artificial	23	2.21 $\pm$ 0.47	2.17 $\pm$ 0.24	1.99 $\pm$ 0.30	5.30	0.07
On-bouts/h	Real	10	3.63 $\pm$ 0.32	3.94 $\pm$ 0.46	4.05 $\pm$ 0.33	0.29	0.23
	Artificial	23	3.60 $\pm$ 0.24	3.58 $\pm$ 0.23	3.84 $\pm$ 0.27	0.29	0.86

Table A2.3. Red-winged blackbird behaviour at nests tested with artificial cowbird eggs according to manipulation type during the “pre-cowbird egg” (PRE), “cowbird egg” (CBE) and “post-cowbird egg” (POST) observation periods. Values are reported as means  $\pm$  SE.

Variable	Manipulation type	n	PRE	CBE	POST	Friedman's ANOVA	
						$\chi^2$	p
Percentage of time present at nest	Add	15	82.77 $\pm$ 8.99	85.34 $\pm$ 8.70	85.75 $\pm$ 6.73	0.93	0.63
	Switch	8	79.99 $\pm$ 7.00	82.13 $\pm$ 6.89	80.57 $\pm$ 5.18	3.25	0.20
Peers/h present at nest	Add	15	4.54 $\pm$ 1.81	4.53 $\pm$ 2.17	4.38 $\pm$ 1.59	0.93	0.63
	Switch	8	5.11 $\pm$ 2.12	5.20 $\pm$ 2.36	5.61 $\pm$ 1.91	1.00	0.61
Percentage of time peering when at nest	Add	15	0.25 $\pm$ 0.14	0.36 $\pm$ 0.38	0.28 $\pm$ 0.18	1.60	0.45
	Switch	8	0.28 $\pm$ 0.19	0.24 $\pm$ 0.09	0.27 $\pm$ 0.08	0.25	0.88
Probes/h present at nest	Add	15	8.14 $\pm$ 4.70	7.82 $\pm$ 2.93	6.80 $\pm$ 2.42	4.93	0.09
	Switch	8	6.13 $\pm$ 2.15	7.76 $\pm$ 2.49	6.07 $\pm$ 1.86	4.75	0.09
Percentage of time probing when at nest	Add	15	2.25 $\pm$ 1.91	2.22 $\pm$ 1.09	2.18 $\pm$ 1.63	2.80	0.26
	Switch	8	2.13 $\pm$ 2.95	2.06 $\pm$ 1.28	1.65 $\pm$ 0.93	3.25	0.20
On-bouts/h	Add	15	3.40 $\pm$ 0.95	3.32 $\pm$ 0.75	3.51 $\pm$ 1.09	0.67	0.72
	Switch	8	3.96 $\pm$ 1.46	4.08 $\pm$ 1.51	4.45 $\pm$ 1.46	0.75	0.69

Table A2.4. Red-winged blackbird behaviour at nests tested with real cowbird eggs according to manipulation type during the “pre-cowbird egg” (PRE), “cowbird egg” (CBE) and “post-cowbird egg” (POST) observation periods. Values are reported as means  $\pm$  SE.

Variable	Manipulation type	n	PRE	CBE	POST	Friedman's ANOVA	
						$\chi^2$	p
Percentage of time present at nest	Add	5	86.90 $\pm$ 2.47	85.47 $\pm$ 2.74	83.08 $\pm$ 2.70	7.60	<b>0.02</b>
	Switch	5	85.71 $\pm$ 1.86	84.94 $\pm$ 1.53	79.06 $\pm$ 2.77	3.60	0.17
Peers/h present at nest	Add	5	4.05 $\pm$ 0.59	4.52 $\pm$ 0.79	5.24 $\pm$ 1.03	4.80	0.09
	Switch	5	4.75 $\pm$ 1.07	4.67 $\pm$ 0.56	5.43 $\pm$ 0.56	1.60	0.45
Percentage of time peering when at nest	Add	5	0.22 $\pm$ 0.04	0.38 $\pm$ 0.07	0.38 $\pm$ 0.07	4.80	0.09
	Switch	5	0.23 $\pm$ 0.06	0.30 $\pm$ 0.06	0.33 $\pm$ 0.05	1.60	0.45
Probes/h present at nest	Add	5	7.49 $\pm$ 1.27	8.23 $\pm$ 1.77	7.86 $\pm$ 1.75	0.40	0.82
	Switch	5	5.27 $\pm$ 0.67	6.83 $\pm$ 0.96	6.27 $\pm$ 0.87	4.80	0.09
Percentage of time probing when at nest	Add	5	1.54 $\pm$ 0.39	1.72 $\pm$ 0.37	2.59 $\pm$ 0.45	7.60	<b>0.02</b>
	Switch	5	1.09 $\pm$ 0.25	1.71 $\pm$ 0.34	2.31 $\pm$ 0.74	2.80	0.25
On-bouts/h	Add	5	3.47 $\pm$ 0.48	3.80 $\pm$ 0.56	4.07 $\pm$ 0.66	2.71	0.26
	Switch	5	3.79 $\pm$ 0.46	4.09 $\pm$ 0.78	4.04 $\pm$ 0.23	0.74	0.69

